

## **SUMMARY OF RESEARCH REPORT**

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Principal Investigator: Richard N. Zare

Institution: Stanford University

Address: Department of Chemistry  
Stanford, CA 94305-5080

Telephone: (650) 723-3062  
Fax: (650) 725-0259  
E-mail: zare@stanford.edu

# **Final report, Search for Unique Biomarkers in ALH84001**

## **Aims and objectives – funding period 06/01/97 to 05/31/99**

Four goals were outlined for this project. These were: [1] to reproduce the measurement of polycyclic aromatic hydrocarbons (PAHs) in ALH84001 with both a higher spatial resolution and sensitivity than has been previously reported (Thomas 1995); [2] to extend such measurements to include other members of the Martian SNC (Shergottites, Nahklites, and Chassigny) meteorite clan, in particular the Antarctic Martian meteorite EETA79001; [3] to address issues of potential organic contamination, because at present very little is known about the effect of terrestrial weathering in the Antarctic environment as it pertains to perturbing an indigenous organic distribution within a meteoritic matrix; and [4] to diversify the range of organic compounds studied to include species that can serve as unique biological markers – “molecular fossils” – derived from once living organisms.

In order to achieve this, three specific goals were outlined for the funding period 06/01/97 to 02/28/98. They were: [1] to investigate the effects of terrestrial weathering and organic contamination of meteoritic samples collected from Antarctica; [2] to reproduce and extend upon the measurements of PAHs in ALH84001 reported by Thomas et al. 1995 and McKay et al. 1996 with the aim of establishing or refuting the indigeneity of these species; and [3] to extend the analysis of organic compounds in ALH84001 and EETA79001 to address compounds that are considered to be more biologically relevant than PAHs. All three were successfully accomplished, as detailed in the previous performance report. In brief, however, the results achieved were to establish that the PAHs found in ALH84001 were indigenous and not due to contamination, and to determine that a novel and sensitive technique in meteoritic work, capillary zone electrophoresis (CE), could indeed detect amino acids, a potential class of biomarker.

In the latter funding period of 03/01/98 to 05/31/99, work has concentrated on extending the investigation to amino acids and on comparing the results obtained for these molecules with PAH distributions. For reasons detailed later, amino acids were chosen as good candidates for biomarkers. The specific aims for this period were therefore [1] to develop a protocol where molecules of interest could be detected in the laboratory at concentrations relevant to those found in meteoritic material, [2] to develop a reliable method for identification of these materials, [3] to establish a protocol where these materials could be detected in meteorite material, [4] to ensure that no contamination was introduced during the investigation, and [5] to compare the results obtained with PAH distributions from the same samples. All these goals have successfully been accomplished.

We have developed protocols for investigating the amino acid content of meteoritic material using liquid extraction (water or water + acid) followed by detection using a derivatization scheme and capillary zone electrophoresis (CE). This was achieved utilizing the Murchison meteorite, which was chosen for various reasons. Firstly, it is known to contain organic compounds, with 0.6  $\mu\text{mol}$  of amino acids (Cronin 1983). It is an abiotic ‘benchmark’, meaning that all the organic molecules present are known to have been synthesized abiotically (Anders 1973, Cronin 1989), allowing the abiotic distributions to be characterized. It is well characterized by other workers allowing good comparison of results obtained in this laboratory (e.g. Shock 1990 and references therein), and it is available in relatively plentiful supply, allowing for repeat experiments to be performed in order to optimize a given technique. The results gained are in good agreement with previous results and we are confident that no

contamination is being introduced during the investigation process. This protocol is now available for use on sample sizes on the order of a few hundred mg or less, and for extension to other meteorites. For future reference a sample of meltwater from the Antarctic has been tested for amino acids. Three peaks were detected, none of which corresponded to the twenty terrestrial amino acids.

CE coupled to laser-induced fluorescence (LIF) detection allows very small sample size injection onto the column ( $\approx 60\text{nL}$ ) with ultimate detection limits on the subattomole scale (Takizawa 1998). This has very good potential for investigation of extremely small sample sizes such as are available for the rarer meteorites as long as the molecules of interest are extracted in sufficient numbers. Extraction results in the molecules of interest being transferred to the liquid extract, leaving behind a solid residue which is available for investigation using the Micro Laser Desorption Laser Photoionisation Time-of-Flight Mass Spectrometry ( $\mu\text{L}^2\text{MS}$ ) technique. The extraction, with the possible exception of the lower mass PAH naphthalene, appears to leave the PAH distribution intact for investigation. This is as would be expected given the hydrophobic nature of these molecules and the polar nature of the extraction solvent, and it is likely that any loss of naphthalene is due to it having been driven off rather than passing into solution.

Some experiments have also been performed with the aim of detecting amino acids without prior derivatization using 118-nm ionization and detection by mass spectrometry. This has been achieved with a certain amount of fragmentation, but not to the sensitivity attained using CE.

## Choice of amino acids as biomarkers

The goal of this work has been to develop a technique which would allow detection of amino acids in Martian meteorites. Amino acids are considered biomarkers due to their role in terrestrial biochemistry. However, they may also be synthesized abiotically. There are two possible methods for introduction of abiotic amino acids to the surface of Mars. Firstly, they may be delivered by infall of carbonaceous meteorites in which they are abundant (Cronin 1983, Chyba 1992). Secondly, amino acids are thought to have been synthesized prebiotically; model systems give high amino acid yields (Miller 1993). The early conditions on Mars were very similar to those on the prebiotic Earth (Pollack 1987) so amino acids may well have been produced *in situ*. Hence, amino acids of some sort could well be present in the Martian environment. While the simple presence of amino acids would not act as a biomarker, two points would count in that direction. Either a distribution of amino acids which incorporated only the known biological amino acids and/or the presence of polymerized amino acids, even short-chain dimers or trimers, would imply the presence of fossil protein fragments and constitute evidence for relic biogenic activity.

There have been previous attempts to detect amino acids in Martian soil material; in 1976 the Viking landers surface soil samples were studied using gas chromatography – mass spectrometry (GC-MS). No amino acids were detected; however this was later attributed to the high UV flux incident on the Martian surface, combined with the presence of free oxygen and titanium (IV) oxide, resulting in high removal rates for these compounds (Bada 1995). However, these conditions are valid only on the surface and it is possible that amino acids are preserved at a small depth in the Martian crust, and also in meteorites ejected from that crust.

## Detection of $\alpha$ -amino acids in meteoritic material using CE

Previous methods of investigation of amino acids in meteoritic material have centered on Gas Chromatography – Mass Spectrometry (GC-MS) and high-performance liquid chromatography (HPLC). GC-MS has a sensitivity shortcoming in that in order to ionize molecules for detection a significant proportion is subject to fragmentation. More significantly, both techniques consume a relatively large amount of sample, in the 1 to 15 g range. For small and/or rare samples where sample size is extremely limited, such as the Martian meteorites, this is a limitation.

In order to examine amino acids and yet deal with small sample sizes, we have introduced the use of CE to meteorite analysis. This is an extremely sensitive technique which has been shown to detect subattomole ( $10^{-18}$  mol) amounts of amino acids (Cheng 1988). The  $\mu\text{L}$  scale of sample injection coupled with the low absolute number of molecules required for sample detection gives this technique good potential for use with small samples or samples with low amino acid concentration.

Fig. 1 shows the principle of CE (Beckman, 1994). A liquid sample containing extracted derivatized amino acids is injected hydrodynamically for an optimized injection time (typically 5s) into a 57cm-long silica capillary with an internal diameter of  $50\mu\text{m}$ . Shorter injection times do not allow for introduction of sufficient sample onto the column, whereas longer injection times typically lead to broader peaks and a concomitant loss of resolution. A potential is applied along the length of the capillary, leading to separation of compounds with differing electrophoretic mobilities. They are then detected as they pass a transparent window in the capillary using LIF.

CE sample preparation involves a certain amount of sample handling in order to extract and derivatize the molecules of interest. Details of the extraction are given in appendix I but, in brief, it involves water and/or 6M HCl extraction at  $\approx 100^\circ\text{C}$  for periods of 24 to 48 hours. Similar procedures have been shown to result in the extraction of amino acids (e.g. Kvenvolden 1971, Lawless 1974), a result which is validated here.

Derivatization of the amino acids is by fluorescein isothiocyanate (FITC), a fluorescent agent whose acid derivatives have a strong absorption matching the 488nm line of the argon-ion laser used to induce fluorescence. Details of the FITC-amino acid recipe and derivatization scheme along with results for the separation of laboratory test solutions are given in detail in appendix II; these reaction conditions are simple and reproducible, and the detection limits for FITC-amino acids using infinite-dilution studies are ca.  $10^{-11}$  to  $10^{-12}$  M.

Before use the glassware involved is subject to an extensive cleaning regime, first in a base bath (KOH in isopropanol) followed by heating in a furnace at  $550^\circ\text{C}$  for 24 hours. Details are given in appendix I. This cleaning process has been developed to ensure contamination of the sample does not occur. Although extraction and derivatization have the potential to result in contamination, careful comparison with blanks which have been subject to the same handling as the samples of interest shows that contamination is not introduced via these processes. This may be seen by examining Fig. 2, which clearly indicates that under the current regime the extraction does not result in contamination. The advantage of such an extraction technique is that results are not limited to a possibly anomalous surface but instead give details on a good proportion of the molecules contained within the rock matrix. Typical results are shown in Fig. 3 and it may clearly be seen that several additional peaks due to amino acids are present. Glycine, alanine and valine, thought to be amongst the most abundant amino acids in Murchison (Shock 1990), are present. Norleucine, a non-protein amino acid, has also been

identified. This supports an abiotic source of the amino acids determined here and is also consistent with the peaks identified being indigenous to Murchison and not due to laboratory or other contamination. In Fig. 4 the Murchison spectrum is labelled with arrows whose direction indicates whether that peak has been increased or decreased from the water blank. Only side FITC peaks are diminished (this is an additional method of characterizing these peaks). All other peaks are species extracted from Murchison.

The effect of concentration on a laboratory solution is shown in Fig. 5. Concentration of the liquid extract improves sensitivity although it may be seen that amino acids are clearly detectable in plain water extract with no concentration. Alteration of the pH of a solution alters the migration times of particular peaks, and hence the use of acid extraction somewhat alters the amino acid peaks. A comparison of the normal water extract and acid extract spectra is shown in Fig. 6. In this case an aliquot was taken after the water extraction before adding acid and taking the acid extraction to completion. The two spectra offer a direct comparison of the two methods on the identical sample. It may be seen that acid extraction enhances the number and intensity of peaks present in the region of interest, and that the change in pH has altered the migration times of the peaks (the main FITC peaks in each case are marked with a dot).

The use of FITC as a derivatization agent allows sensitive detection of amino acids in a convenient spectral region, as discussed above. There are, however, some properties of FITC which require careful interpretation of results. Plain FITC has a very intense main peak and a number of side bands (Brumley 1997, Lalljie 1995) which are of comparable intensity to those seen for amino acids in real samples. It also degrades both photochemically and simply over time in solution, resulting in the increase in intensity of some side peaks and the decrease in intensity of the main peak and the remainder of the side peaks. We have determined that these problems can be overcome with the careful monitoring of the equivalent blank systems and with time-dependent analysis of the derivatization reaction, as shown in Fig. 7 which illustrates 24-hour monitoring of FITC in plain HPLC water. This allows characterization of the time-dependent behavior of the FITC peaks so that they may be discounted in analysis, even where they are larger than peaks of interest. Fortunately, the FITC side peaks occur mainly out of the region where the amino acid peaks appear; in addition, amino acid peaks only increase in intensity as the derivatization reaction proceeds to equilibrium, ca. 24 hours, so peaks whose intensity decay may immediately be discounted. Photochemical degradation of FITC is easily avoided with the use of amber vials and simple light shields. Although the system is complex, it has been fully characterized using these monitoring conditions and careful data analysis. Different reaction conditions are often reported in the literature for FITC derivatization, but since these only report the peaks at one time this does not easily allow comparison between different methods. The 'timelapse' spectra gained here, however, should allow such comparison. Fig. 8 shows a typical monitoring sequence over time for Murchison extract in the region of interest. The Valine peak at ca. 11 minutes may clearly be seen to increase in size over this time period.

The intrinsic fluorescence of the FITC peak itself is actually a fortuitous occurrence, since it acts as an internal standard. This allows peak identification according to

$$\frac{t'_{AA \text{ PURE}}}{t'_{FITC \text{ PURE}}} = \frac{t''_{AA \text{ MIXTURE}}}{t''_{FITC \text{ MIXTURE}}}$$

where  $t$  = migration time of a given peak,  $t'$  AA PURE = the migration time of the amino acid in a lab test solution containing that amino acid,  $t'$  FITC PURE = migration time of the main FITC peak in that solution, and  $t''$  AA MIXTURE and  $t''$  FITC MIXTURE = the migration times of the unknown amino acid peak and the main FITC peak in a mixture/real-life sample respectively. In addition, it allows for semi-quantitative comparison between spectra taken at an equivalent reaction time via peak normalization.

Finally, in order to evaluate the amino acid content of Antarctic ice for future reference, a sample of meltwater was derivatized and investigated in the same manner as the water extracts. This environment is an important source of meteorites and is purportedly pristine with respect to contamination of meteorites with terrestrial material; nevertheless any amino acids present in the meltwater offer the possibility that they may percolate through extraterrestrial samples (McDonald 1995). Fig. 9 shows the resultant spectrum. There are three distinct peaks, none of which correspond to the 20 common terrestrial amino acids, and these three peaks are therefore due to amines or non-protein amino acids in the ice. These peaks may constitute markers for terrestrial contamination.

## Investigation of PAHs in pre- and post- extracted samples using $\mu\text{L}^2\text{MS}$

PAHs are a class of molecules which are widespread throughout both the terrestrial and extraterrestrial environment (Allamandola 1985, Leger 1984). Fig. 10 shows the structure of some common PAHs. Their importance, since they may be manufactured by a number of both biological and nonbiological mechanisms, lies in the type of distribution rather than their simple presence (Blumer 1976, Friedmann 1970, Oro 1966, Wakeham 1980).

In order to determine the effect of the extraction techniques on the PAH component of the organic load contained in Murchison meteoritic material,  $\mu\text{L}^2\text{MS}$  spectra were taken of pre-extraction and post-extraction Murchison samples. The  $\mu\text{L}^2\text{MS}$  technique is described in more detail in the previous progress report, but briefly it involves the laser desorption of molecules such that they are moved intact and neutral into the gas phase, followed by selective ionization of the PAHs in the desorption plume. This is illustrated in Fig. 11. Fig. 12 depicts the  $\mu\text{L}^2\text{MS}$  instrument. It is observed that the depletion of the PAH distribution after both water (see Fig. 13) and subsequent acid extraction (see Fig. 14 and Fig. 15) is minimal, regardless of the IR wavelength used for desorption. The same results are apparent for both  $\text{CO}_2$  10.6  $\mu\text{m}$  desorption and Er:YAG 2.94  $\mu\text{m}$  desorption. There is a slight change in the relative ratios of some of the peaks although not to an extent which is outside the heterogeneity of Murchison itself. There may be a slight depletion of naphthalene which has a relatively low molecular weight compared to the other PAHs, but given the low solubilities of PAHs as given in Table 1 and given the investigation into PAH motility into water performed during this project (see previous report) it is unlikely that the PAH has been extracted into the liquid phase but instead that a small proportion has been driven off during the heating phases of the extraction procedure.

## Conclusions for CE and $\mu\text{L}^2\text{MS}$ work

In summary, CE derivatization schemes have been developed and derivant characterization has been performed such that amino acids may reliably be detected in solutions which are of the concentrations likely to be found in real-world samples. This has included characterization of the complex time-dependant derivatization scheme. A scheme to extract amino acids from meteoritic material has been successfully developed, and we are confident that no significant amino acid contamination is being introduced during the extraction process.

$\mu\text{L}^2\text{MS}$  has been performed on both water- and acid-extracted samples with the result that the water-insoluble PAH fraction of organics is left essentially intact, with variations to within the known heterogeneity of Murchison, by the extraction processes, with the possible exception of the low molecular weight PAH naphthalene. This result has been replicated using two separate desorption wavelengths.

## Work performed at SRI International with 118-nm ionization mass spectrometry

Three main topics were considered in the period June 1998-May 1999 :

1. Detection of organics on solid samples (meteorites, CV3, Murchison, and coal);
2. Assessment of nonresonant 118-nm ionization mass spectrometry for detection of amino acids (fingerprints, sensitivity, reproducibility) and comparison with EC results;
3. Detection of amino acids in extract from meteorites (Murchison).

## Experimental set-up

The experimental work carried out in the period June 1998-May 1999 has been performed using an apparatus described in published papers (Pallix 1994, Ayre 1994) and in the previous reports. The conditions may be summarized as:

Spectrometer: ToF reflectron,  $M/\Delta M=800$ )

Base vacuum:  $1-2 \times 10^{-8}$  mbar

Primary beam: laser beam (ns Nd:YAG, 532 nm)

Ionization beam:  $\lambda=118$  nm,  $E=10.5$  eV, 9<sup>th</sup> harmonic Nd:YAG (ns) produced by tripling the 3<sup>rd</sup> harmonic in a Xe/Ar cell

In this phase of the project, the wavelength used for desorption has been changed from 355 nm to 532 nm. As expected, this change increased the signal intensity by a factor of about 20. Fig. 16 shows two 118-nm ionization spectra acquired using 355 nm and 532 nm laser desorption respectively from a mixture of PAHs (naphthalene, acenaphthene, phenanthrene, anthracene, fluoranthene) dried on a silicon substrate.

## Results

### Detection of organics on solid samples

Three samples were utilized for analysis and testing of the capabilities of 118-nm post-ionization analysis. Two of them were meteorites, C2-Murchison and CV3, while the third was a terrestrial sample of coal.

Fig. 17 shows a Surface Assisted Laser Ionization (SALI) spectrum acquired with 532 nm desorption and 118-nm postionization from the C2-Murchison sample. Table 2 gives tentative identifications of the major peaks. The dominant features of the spectra are the peaks produced by sulfur-based compounds and sulfur clusters. Low intensity peaks produced by aliphatic hydrocarbons are present in the low mass range. PAH peaks with significant intensity are present in the spectra; these represent a small subset of those previously detected by the Zare group with  $\mu\text{L}^2\text{MS}$  using a (1+1) REMPI postionization scheme. The peaks at mass 149, 167, 208 and 279 can be produced by poly(dimethylsiloxane) (PDMS) and phthalates (dibutylphthalate). These compounds are commonly present in plastics and could have been introduced as a contamination by the plastic envelope where the samples had been stored.

The 118-nm ionization mass spectra from the Murchison meteorite are in agreement with previous analysis of the same meteorite done by Tracy Tingle et al. in the past (Tingle 1991, 1992) using similar experimental conditions. Ionization with 118-nm light is specific for the detection of sulfur compounds and clusters. Similar compounds were not detected by previous analysis carried out on the same sample by  $\mu\text{L}^2\text{MS}$  which utilizes 266nm ionization. The presence of sulfur in the fragments analyzed by 118-nm ionization was confirmed by Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) analysis and mapping of the samples. The photoionization efficiency for sulfur and its clusters as a function of photon energy has been measured previously using a hydrogen lamp (Berkowitz 1968). Those results show an exponential increase for  $E_{\text{ph}} > 9$  eV. This could explain the extra peaks in 118-nm, 10.5 eV, single photon ionization mass spectra compared to the  $\mu\text{L}^2\text{MS}$  266-nm, 4.67 eV, (1+1) REMPI mass spectra. Furthermore, in using a (1+1) ionization scheme, the detection efficiency of  $\mu\text{L}^2\text{MS}$  for sulfur species could be decreased due to the short lifetime of the sulfur compound excited states, analogous to the effects noted with nitrogen containing aromatic compounds or phosphorus compounds where efficient intersystem crossing leads to high losses from the excited single state (Oser).

Sample CV3 presents comparatively simple 118-nm ionization mass spectra (Fig. 18). Hydrocarbon peaks are present in the low mass range. The peak intensities for the sulfur-related compounds are strongly reduced compared to the results seen for Murchison, in agreement with the low S content in the sample measured by SEM-EDX analysis.

Analysis of the coal sample revealed the presence of aliphatic hydrocarbons, but no PAHs (Fig. 19). Occasionally, the presence of PDMS and phthalates (dibutylphthalate) were detected, suggesting sample contamination due to handling/storage.

No evidence of amino acids in the meteorite samples was found utilizing 118-nm ionization mass spectrometry, in agreement with the results previously obtained using this technique to analyze the

Murchison meteorite presented by Tingle (1991, 1992), despite the known presence of these compounds.

In an attempt to increase the desorption yield, the surface of some meteorite and coal fragments were treated with DHB (2,5-dihydroxybenzoic acid), a matrix commonly used in MALDI (matrix assisted laser desorption ionization) mass spectrometry. It has been reported that this treatment enhanced the sensitivity in ToF mass spectrometry (Wu 1996) and in laser-SNMS (Secondary Neutral Mass Spectrometry) of metals on Si surfaces (Moro 1999). In the two cases the enhancement is produced by different effects: enhanced surface ionization in SIMS, increased adsorption of the laser beam in laser-SNMS. For the samples under investigation the treatment did not produce any significant increase of ion yield.

To complete the characterization of these solid samples some direct ion mass spectra were acquired in the Time-of-Flight-Secondary Ion Mass Spectrometry (TOF-SIMS) mode. In these spectra characteristic peaks of the most stable PAHs (naphthalene, phenanthrene, anthracene, fluoranthene) are observed, together with the characteristic peaks of PDMS and phthalate supporting sample contamination.

### **Assessment of 118-nm ionization mass spectrometry for detection of amino acids**

A set of amino acids (Table 3) was selected to assess the capacity of 118-nm desorption to detect these species. Fig. 20 shows the spectra produced by a 2 $\mu$ l drop of 10<sup>-2</sup>M solutions (in H<sub>2</sub>O) dried on a silicon substrate (<10nM).

With the exception of the adenine phosphate salt, for all the amino acids the base peak is the (M-45) fragment that corresponds to the loss of the carboxyl group. For the adenine phosphate salt, the adenine parent peak produced by the loss of the phosphate group is the base peak. The parent peak is also present at mass 75 for glycine. For l-serine together with the base peak corresponding to the loss of the carboxyl group, the peak at mass 75 corresponding to the loss [HO-CH<sub>2</sub>] characterizes the compound. In all cases, these fragment patterns for the investigated amino acids allow the unambiguous identification of the amino acids. An attempt to increase the base peak intensity and/or to obtain the parent peak by using mixed amino acid/DHB solutions has been unsuccessful.

Fig. 21 shows the spectra acquired from drops (1 $\mu$ l) of solutions at different molarity of glycine dried on silicon substrate. A few observations can be drawn from these spectra:

- 100 pmol of compounds are easily detected by 118-nm ionization mass spectrometry (Fig. 21c) and a detection sensitivity limit of 5-10 pmol can be expected under these experimental conditions;
- the 10<sup>-2</sup>M and 10<sup>-3</sup> M solutions give comparable intensity suggesting that the silicon surface cleaning and final condition (hydrophobic vs. hydrophilic) play an important role in signal intensity. The detection limit is partially controlled by how much the solution spreads or beads up on the substrate;
- effective cleaning of the surface is also important to reach high sensitivity since it prevents interference from unwanted contamination that could overlap with the compound peak, hindering any semiquantitative evaluation or confusing the compound identification.

Different cleaning procedures of the silicon substrate have been tested and some results are depicted in Fig. 22. The RCA+NH<sub>4</sub>F regime has been selected for future preparations.

Solutions of 5 amino acids (l-alanine, serine, proline, valine and l-glutamic acid) with molarity ranging from  $10^{-2}$  to  $10^{-4}$  M were analyzed. Fig. 23 shows the spectra acquired from 1  $\mu$ L of each solution dried on a silicon substrate. The amino acids can be unambiguously identified even in the  $10^{-4}$  M solution. The absolute concentration of the amino acids in solution is the same, and the relative peak intensity of the different amino acids is within a factor of 3-4. The relative intensity of the signals is not constant for different acquisitions although this variation does not depend on the solution molarity, but rather on selective and heterogenous crystallization of the sample while drying. Fig. 24 shows the scattering of the ratio of intensity of the base peak of different amino acids to the intensity of the base peak of l-alanine. In the worst case the variation is ca. 50%. This indicates that by spiking an unknown solution by a known amount of a selected amino acids, a *semiquantitative* evaluation of the amount of amino acids content in the sample is possible.

In Fig. 25 the 118-nm ionization mass spectrum of the dipeptide Gly-Ala is depicted. The compound was investigated to establish what kind of information is obtained in the presence of slightly more complex combinations of amino-acids. In the spectrum a number of peaks can be identified as specifically produced by molecular fragmentation, and their identification is suggested in the inset.

### Detection of amino acids in extract from meteorites (Murchison)

A series of preliminary experiments on "simulated samples" have been performed with the simultaneous aims of

- comparing the results given by 118-nm ionization mass spectrometry and CE in terms of consistency and sensitivity of the two techniques, and
- finding ideal experimental conditions for the amino acid extraction. Critical points to successfully accomplish the extraction are efficiency of the process and minimizing accidental contamination during the process.

To simulate meteoritic material, SiO<sub>2</sub> powder had evaporated onto it a lab solution of a mixture of amino acids of a given molarity comparable to the known content of amino acids in Murchison.

Fig. 26 is the summary of one of these experiments. Fig. 26a shows the spectrum obtained from water that had been circulated in the empty extraction column with experimental conditions simulating the extraction process to be used for the meteorites (for 10 hours at 100°C). Many unidentified peaks suggesting the presence of aliphatic hydrocarbons are present in the spectrum. Similar peaks are present in the spectrum of Fig. 26b obtained from the solution collected after an "extraction" run in the same conditions with the pure SiO<sub>2</sub> powder in the column. Finally, the spectrum produced by an extraction run of a simulated sample is shown in Fig. 26c. This simulated sample was produced by evaporating 2  $\mu$ L of a  $10^{-2}$  M mixture of the L-amino acids proline, valine, serine, alanine and glutamic acid on the SiO<sub>2</sub> powder.

The base-peaks of l-alanine, proline and valine are clearly present. The peaks produced by other compounds extracted from the SiO<sub>2</sub> and, by comparison with Fig. 26a, by contamination introduced by the extraction apparatus are also present, with lower, but still considerable, intensity. The "extracted" SiO<sub>2</sub> powder was successively analyzed. The spectrum in Fig. 26d shows the considerable amount of amino acid left on the powder by the inefficient extraction process. In both the extracted solution and the left over SiO<sub>2</sub> powder only three of the five amino acids were detected. (In comparison all five were easily detected performing CE on the extracted solution.)

The results of a similar experiment run after modification of the extraction column are shown in Fig. 27 a) the water collected after a run with no material in the extraction column, b) extraction with SiO<sub>2</sub>, and c) extraction of a simulated sample. Peaks due to contamination (i.e. Na at mass 23 and K at mass 39) or other extracted compounds are still present, although with less intensity compared to Fig. 26, but all five amino acids could be detected in the spectrum.

Two extractions were run with the Murchison meteorite using the same extraction conditions as for the SiO<sub>2</sub> blank. Figures 28 and 29 show that for both the extractions the dominant features of the spectrum are, once again, the sulfur related compound. Na and K contamination also produce strong signals in the spectra. In the low/medium mass range the spectra are populated with peaks with the characteristic periodicity of hydrocarbons. These peaks may overlap with the amino acids' characteristic peaks and make a positive identification impossible.

## Conclusions

A summary of the conclusions that can be drawn from the experiments carried on in this project by using 118-nm ionization mass spectrometry to detect amino acids from meteorites is given below.

- 118-nm ionization mass spectrometry allows an unambiguous identification of amino acids.
- The detection sensitivity of the technique is in the range of 10 pM, that is within a factor 10 of the best detection limits reported using mass spectrometry. This detection limit may be somehow improved by suitable pre-concentration techniques.
- The sensitivity to different amino acids is within a factor of ca. 4.
- The reproducibility of the normalized signals is better than 50 %.
- Semi-quantitative determination of the amount of amino acids in unknown samples would be possible by using spiking methods.
- As is to be expected, the detection sensitivity of mass spectrometry is not comparable with the claimed sensitivity of CE (10 attomol). Given the substantial consistency of the results obtained on the (limited) number of experiments performed, analysis by mass spectrometry would be a useful tool to independently validate EC results, at least when a significant amount of material can be used for extraction.
- The major problem encountered in real samples is preventing contamination.
- The efficiency of the water-only extraction process could be improved since analysis of the extracted powders has shown that a large amount of amino acids remained.
- While in control samples the identification of amino acids is easy, the detection and quantification of amino acids in extracts may be difficult because of the presence and overlapping of other compounds and/or contamination using this technique.

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## Appendix I

*Cleaning* – in order to ensure that the equipment used to extract amino acids is clean, a vigorous regime is utilized to remove any amino acids or other organics. Initially the equipment is soaked in a KOH/isopropanol base bath for 24 hours, following which it is thoroughly rinsed with distilled water and then placed in a furnace at 550°C for 24 hours. The recipe for the base bath is as follows:

Dissolve 480g of NaOH in 480ml H<sub>2</sub>O, with the holding vessel placed in an ice bath. Some stirring is required to ensure full dissolution of the NaOH. This mixture is then diluted in 4L of isopropanol.

*Extraction protocol* – the extraction apparatus is as shown in Fig. 30. The water extraction is performed using 20ml of HPLC water for 24 hours. N<sub>2</sub> is passed during the extraction and the mixture is held to just boiling with a heating mantle. The meteorite sample is prepared via removal of the outer layer by scraping with a surgical scalpel, followed by grinding in a mortar and pestle subject to the same cleaning regime as the extraction apparatus. While attempts were made to suspend the sample using a platinum grid in the vapor phase for vapor extraction, this did not work for practical purposes as the particle size distribution inevitably led to some material being lost into the bottom flask. It is therefore simpler to neglect the suspension cage altogether. Dispensing with its use did not lead to any loss of final peak intensity. In order to avoid cross-contamination, each extraction flask was only used once, although the remainder of the apparatus was cleaned and re-used.

The relevant water extract may be derivatized and sampled directly, or pre-concentrated to dryness and rediluted to a higher concentration than was originally the case before derivatization, with care taken to ensure that any amino acids which have dried on the sides of the evaporation container are sonicated back into solution when redilution takes place. Both the water sample and the powdered meteorite remains may then be subject to acid treatment.

Acid treatment of either the concentrated water extract or of the meteorite remains involves the addition of ca. 20ml of 6M HCl for 24 hours. N<sub>2</sub> is passed during the extraction and the mixture is held to just boiling with a heating mantle. There is a distinct change from a colorless acid to a highly colored yellow extract. This is then evaporated to dryness and rediluted as above. In the case of acid extraction from the solid sample the liquid is centrifuged prior to drying.

## Appendix II

The solutions used at various stages of the investigation process are detailed below.

### Experimentally determined buffered extract recipe for optimum peak intensity:

2.25ml extract  
0.25ml 200mM carbonate buffer  
2.50ml 5mM carbonate buffer  
20 $\mu$ L FITC solution

### 200mM carbonate buffer for sample preparation, pH=9:

50mL distilled H<sub>2</sub>O  
0.7896g Sodium Bicarbonate  
0.636g Sodium Carbonate

### 5mM carbonate buffer for sample preparation, pH=10:

50mL distilled H<sub>2</sub>O  
0.0210g Sodium Bicarbonate  
0.0265g Sodium Carbonate

### FITC derivatizing solution:

10mg FITC  
46.7mL Acetone

### 50mM carbonate buffer for sample separation, pH=10:

50mL D<sub>2</sub>O  
0.1260g Sodium Bicarbonate  
0.1060g Sodium Carbonate

Fig. 31 shows the FITC-amino acid derivatization scheme and Fig. 32 shows the separation of a solution of a test mixture of 21 amino acids. Each amino acid was dissolved in 200mM carbonate buffer at a concentration of  $10^{-4}$ M. The individual solutions were then combined and diluted to  $10^{-6}$ M using 5mM carbonate buffer. 4mL of this mixture was derivatized with 20 $\mu$ L of FITC derivatizing solution and allowed to react for 20 hours in the dark. 15 amino acids give distinct peaks while the remainder coelute with one or more other species. The migration times of this solution have been used as the standard to identify unknown peaks in real-world and extraterrestrial samples.

**Figure 1: Schematic of LIF – Capillary Electrophoresis Instrument**

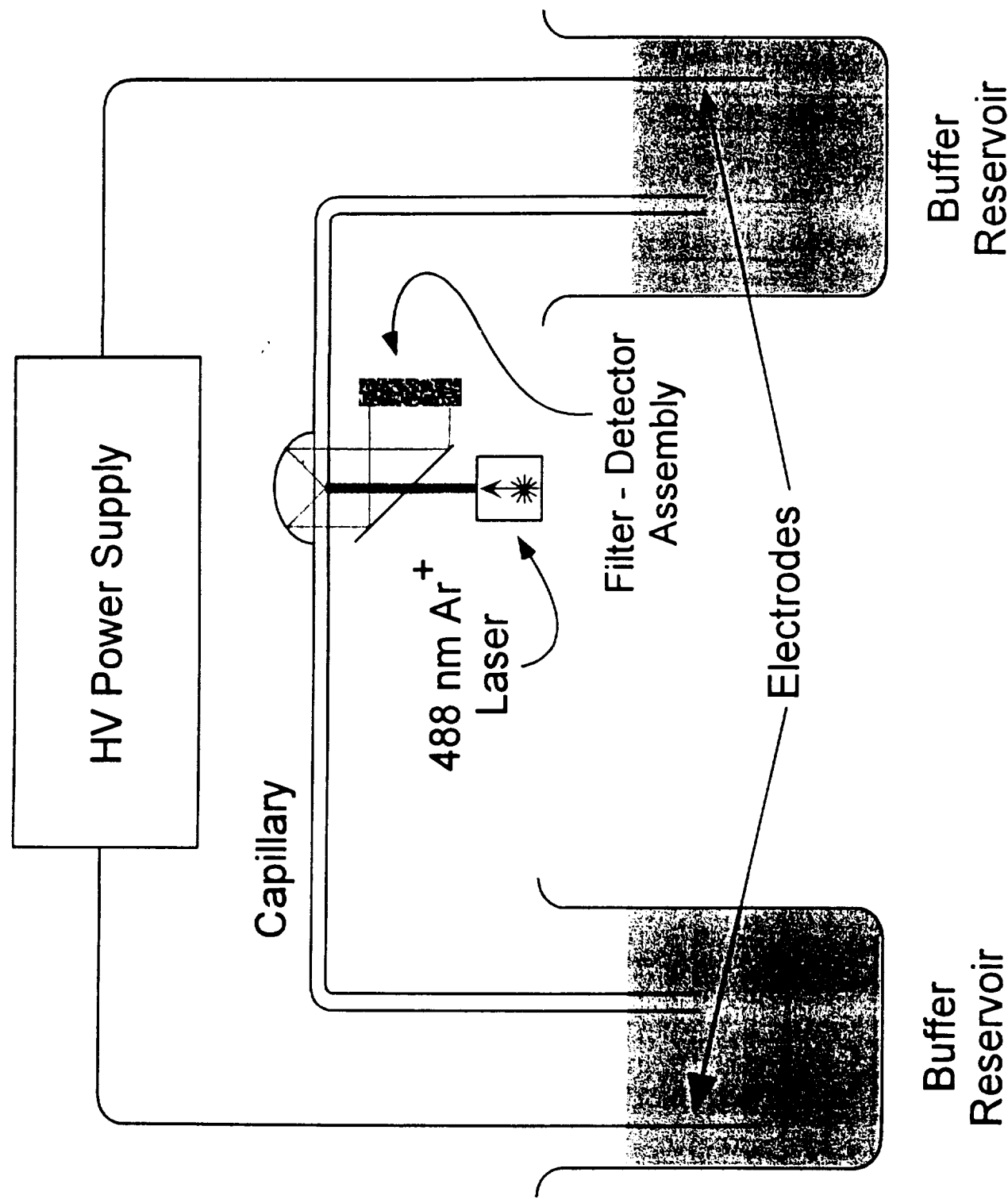
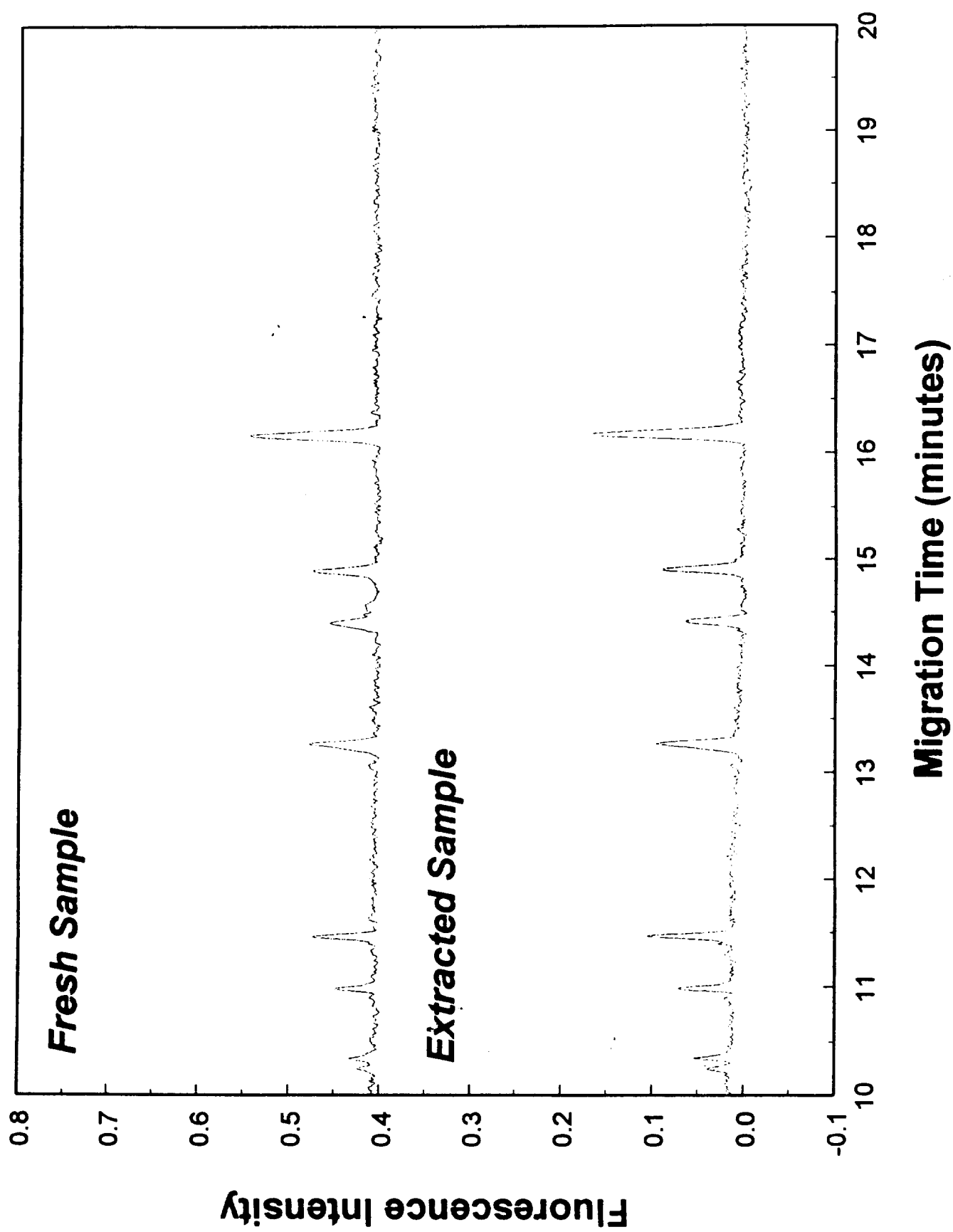
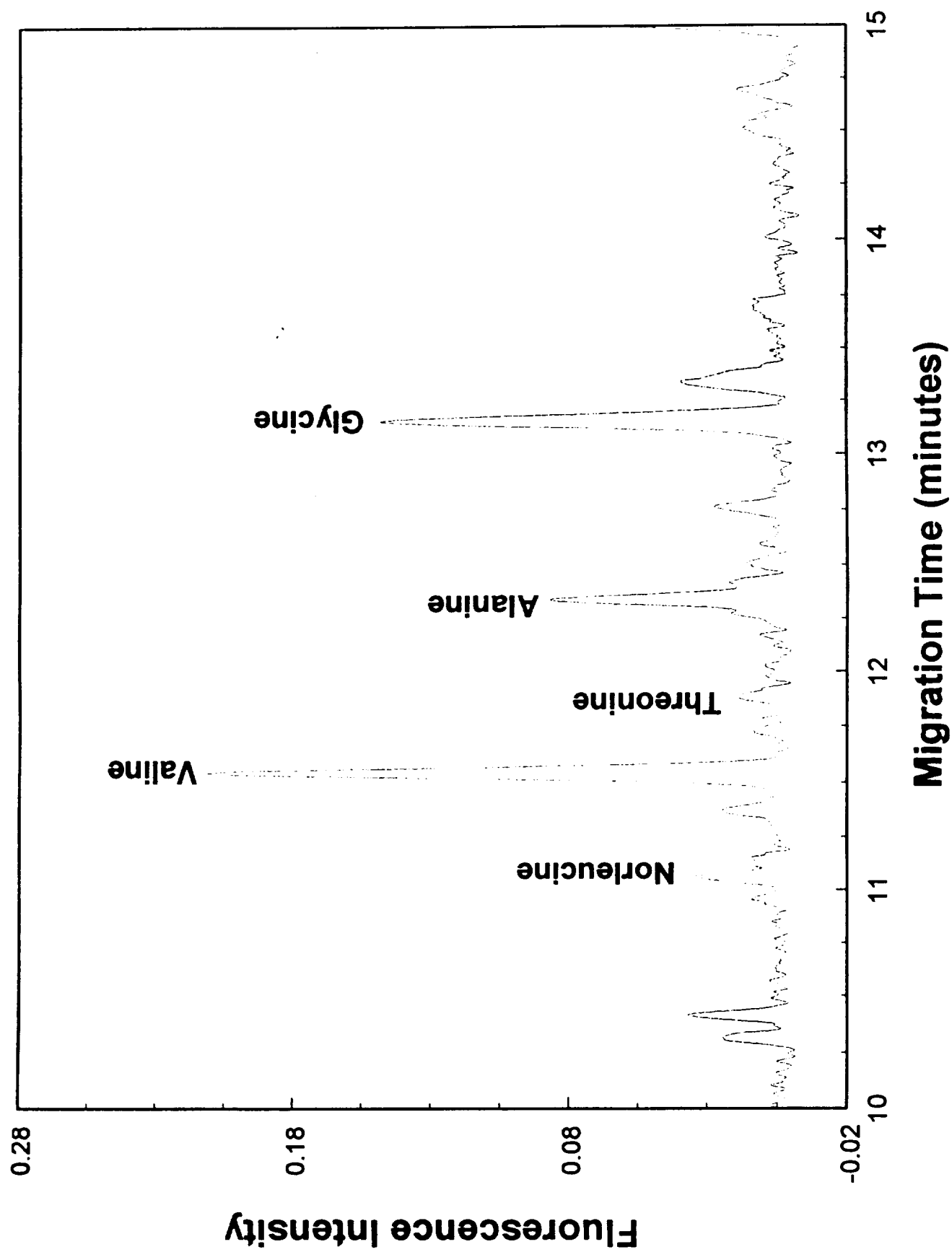


Figure 2: HPLC grade water derivatised by FITC showing region of interest



**Figure 3: Murchison meteorite extract with main peaks labelled**



**Figure 4: Electropherograms of extracted Murchison (dashed) and plain water (solid)**

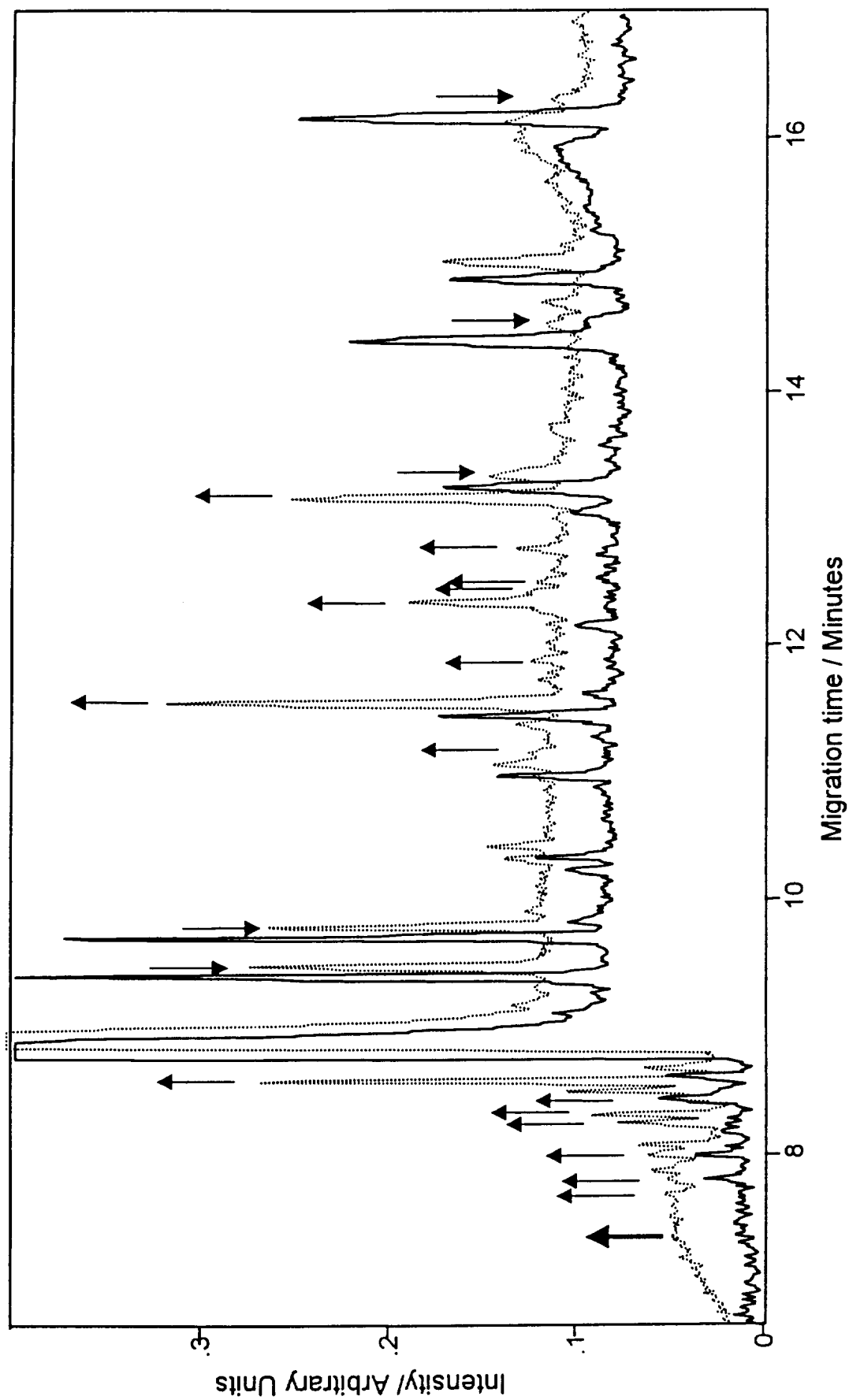


Figure 5: Effect of concentration on peak intensity of laboratory solution of amino acids

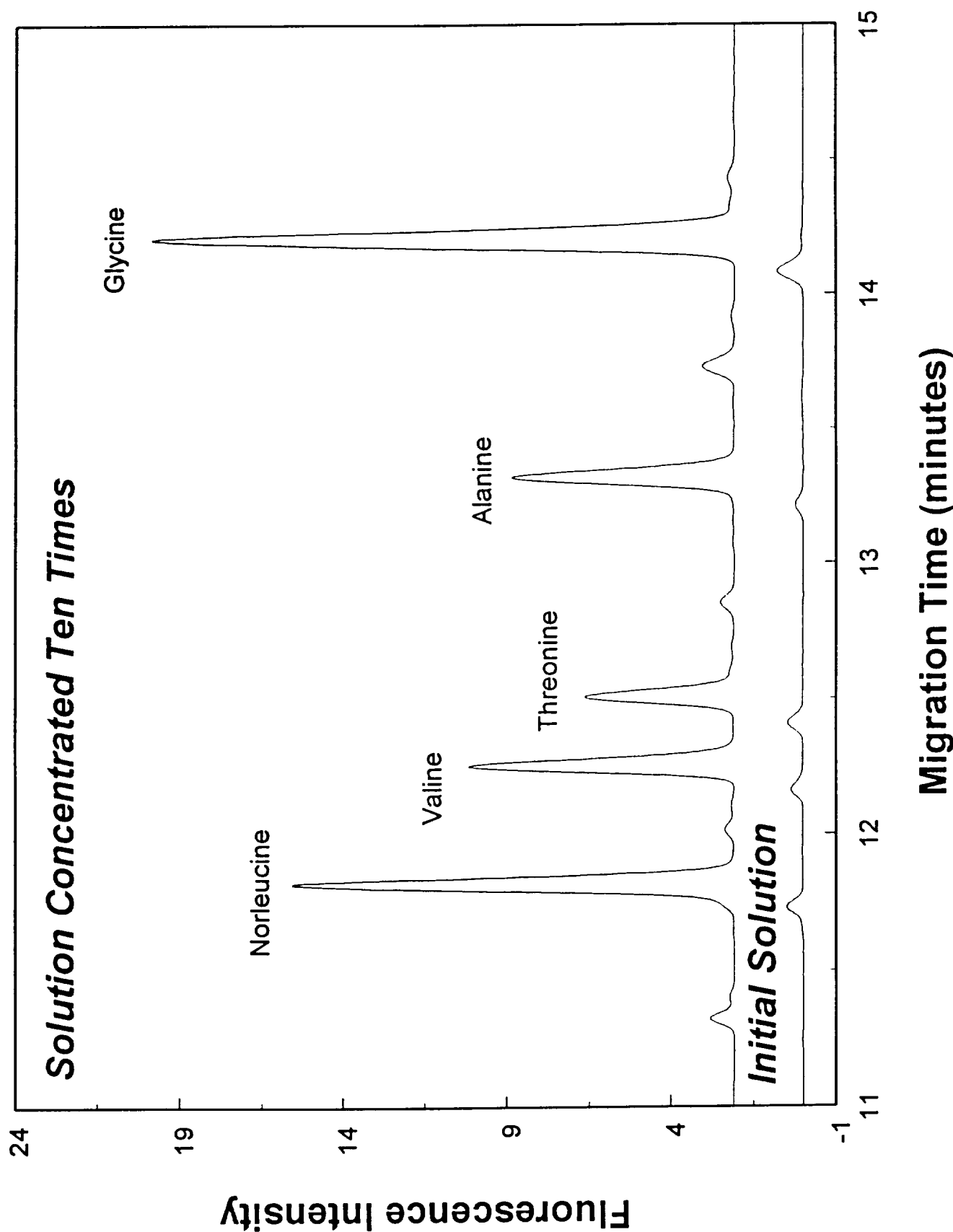
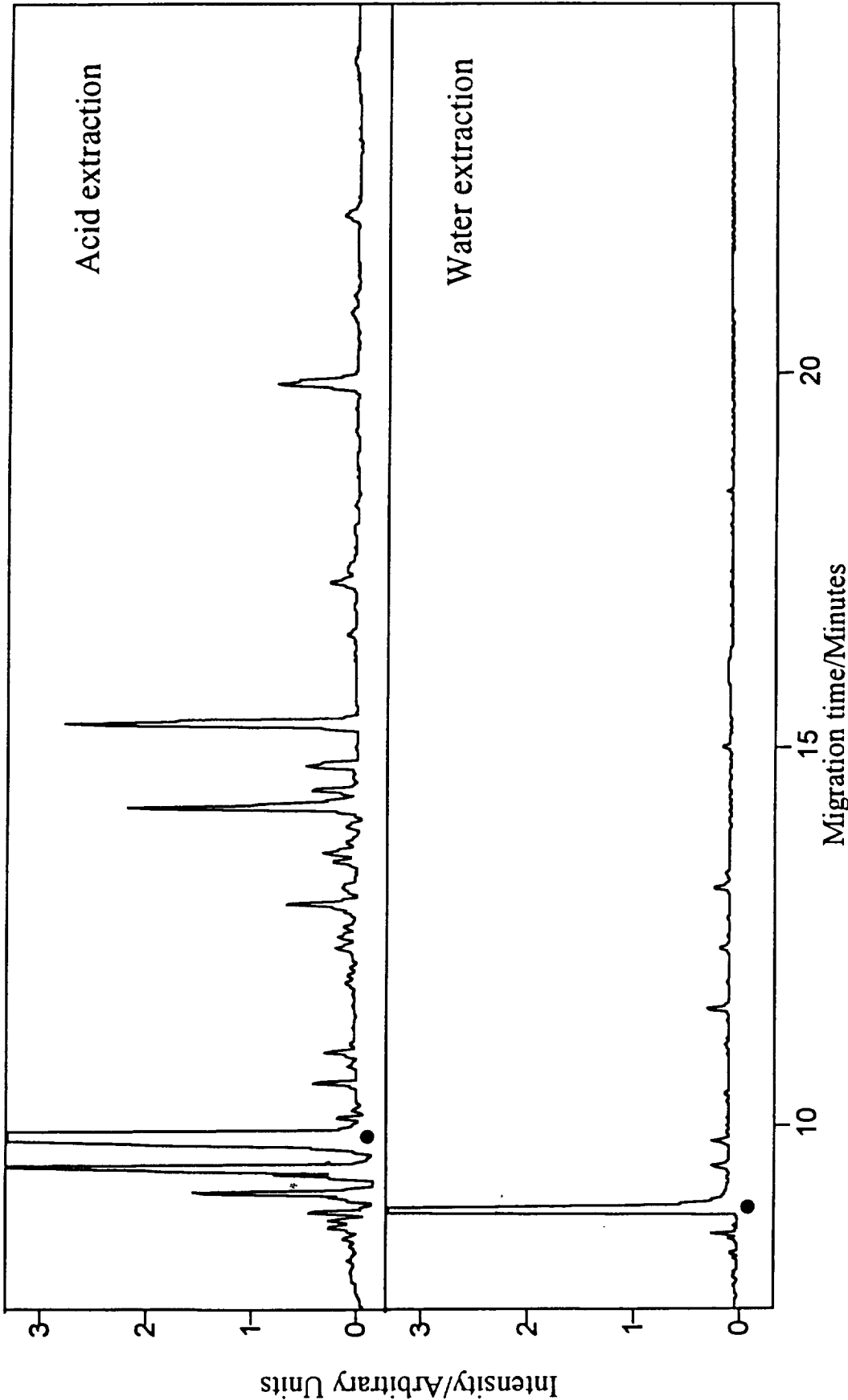
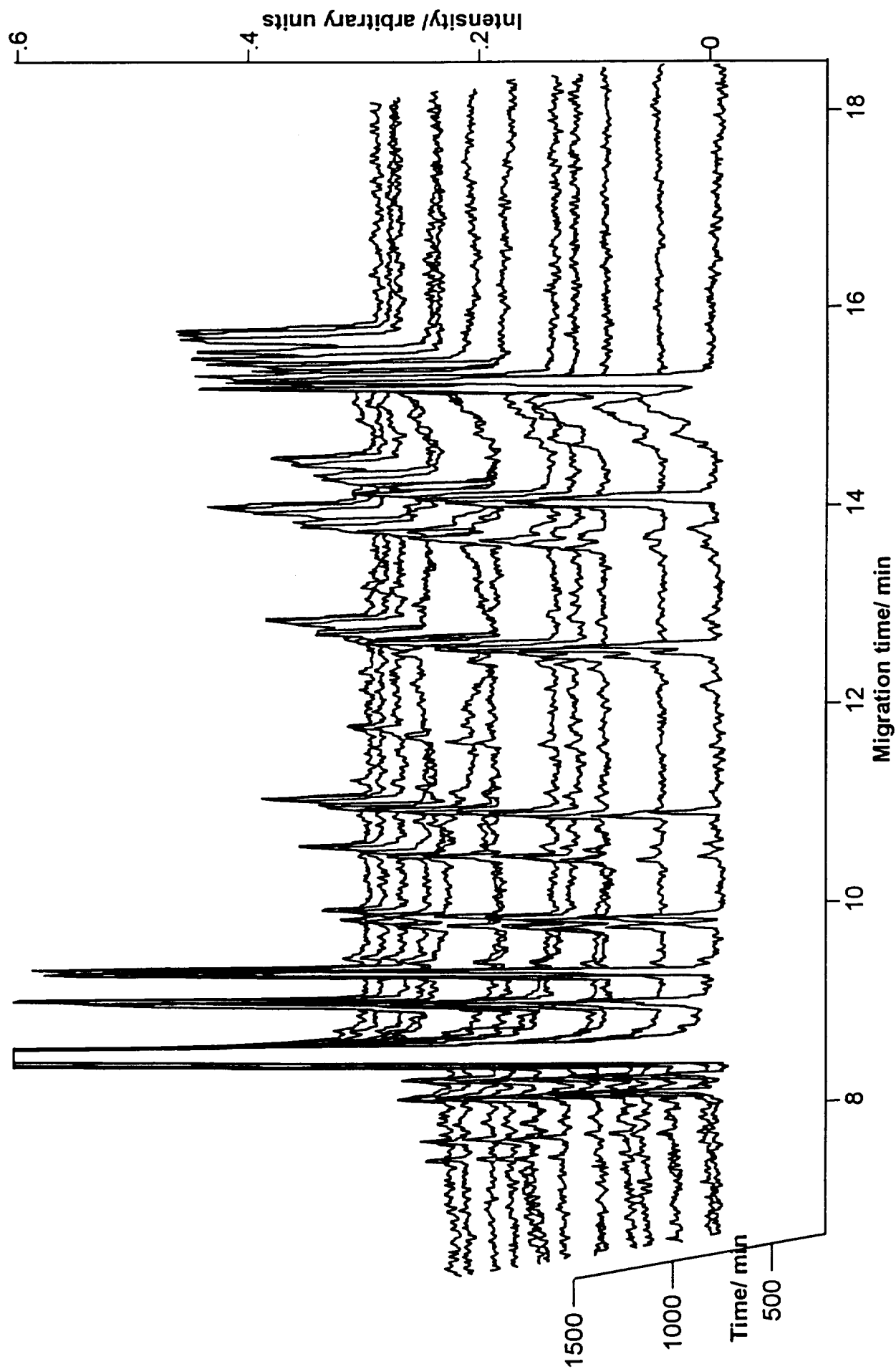


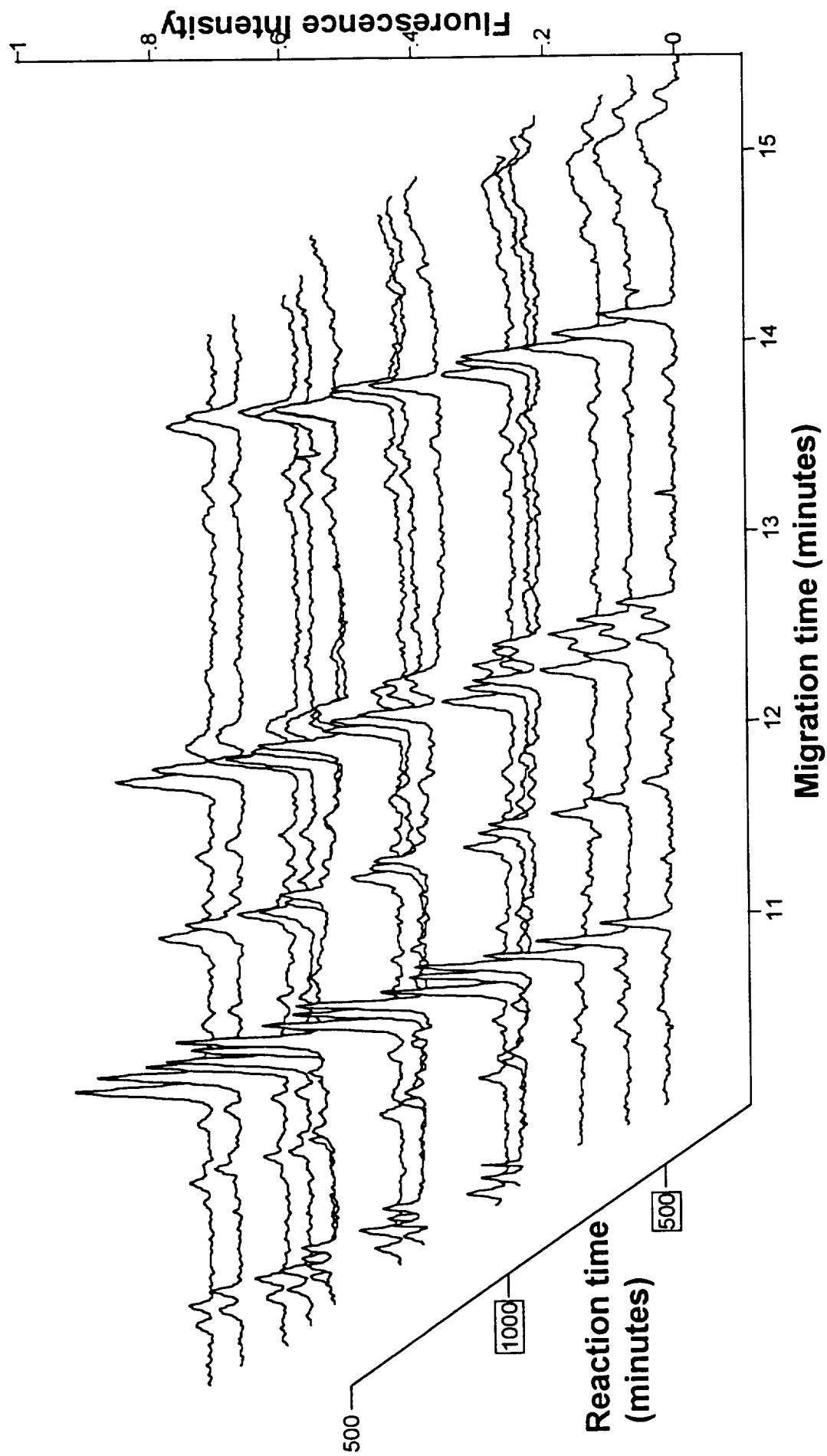
Figure 6: Comparison of electropherograms for acid- and water- extracted Murchison



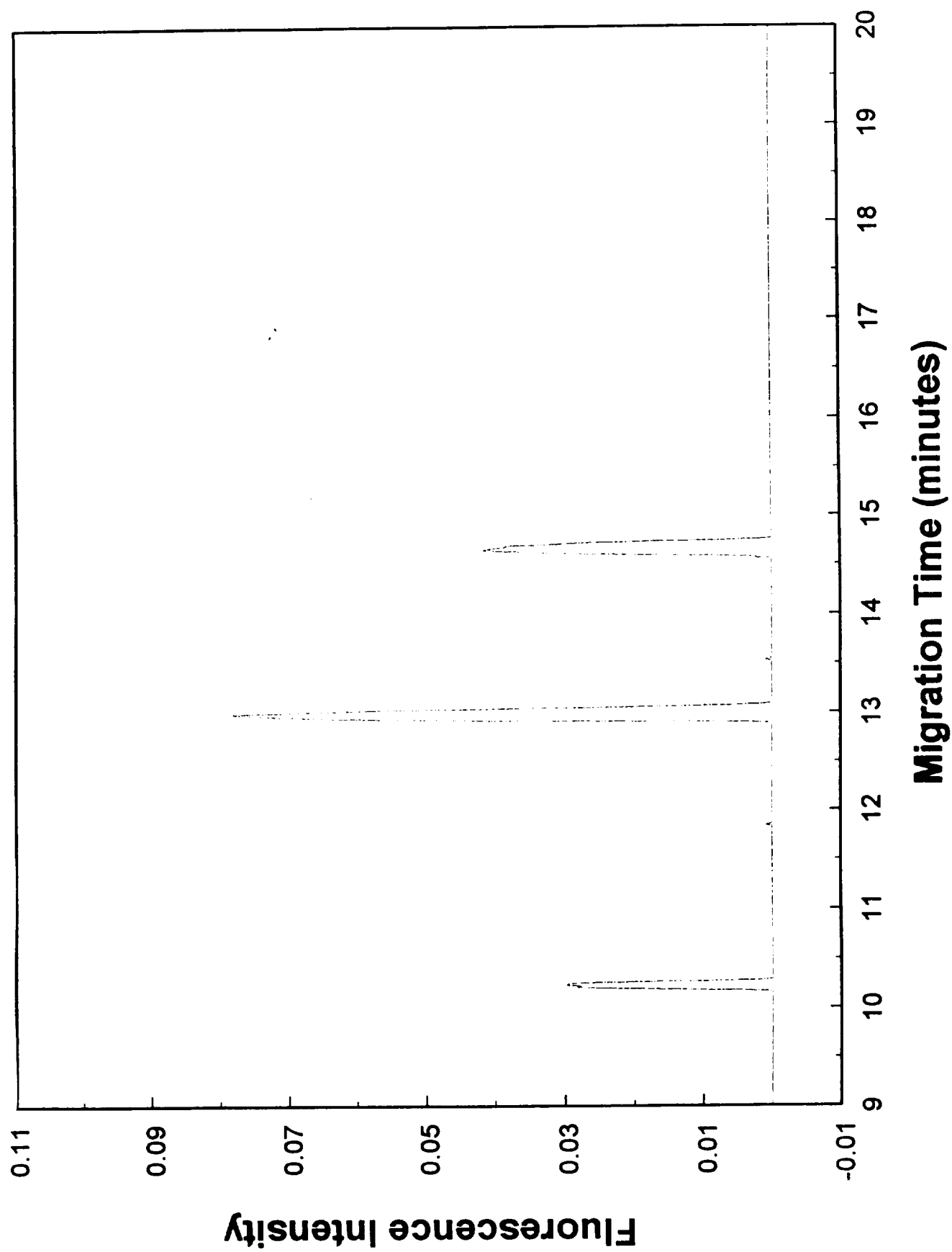
**Figure 7: 24-hour monitoring of FITC in plain HPLC water.  
Electropherograms are started at 90-minute intervals**



**Figure 8: 24-hour monitoring of FITC-derivatized Murchison water extract**

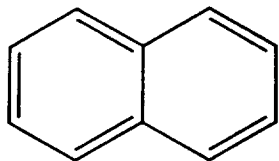


**Figure 9: Electropherogram of Antarctic Ice Meltwater sample**

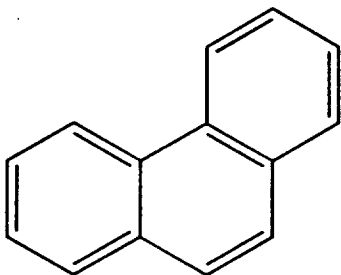


**Figure 10: Structures of some common PAHs**

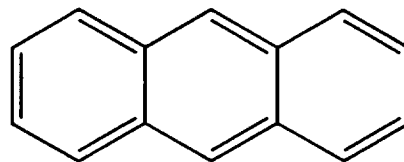
**Naphthalene (128 amu)**



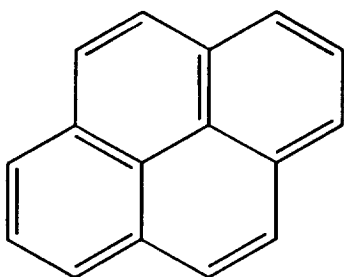
**Phenanthrene (178 amu)**



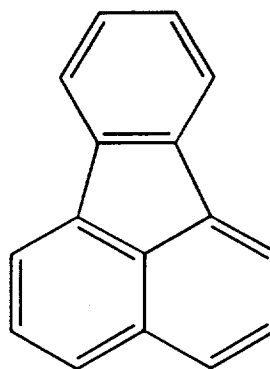
**Anthracene (178 amu)**



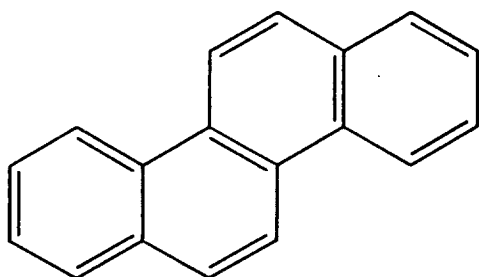
**Pyrene (202 amu)**



**Fluoranthene (202 amu)**



**Chrysene (228 amu)**



**Benzo(a)anthracene (228 amu)**

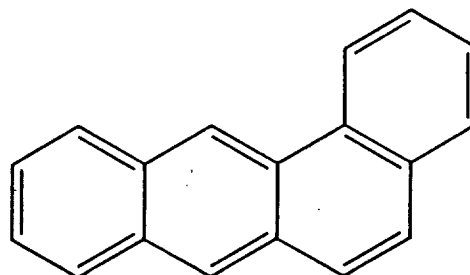
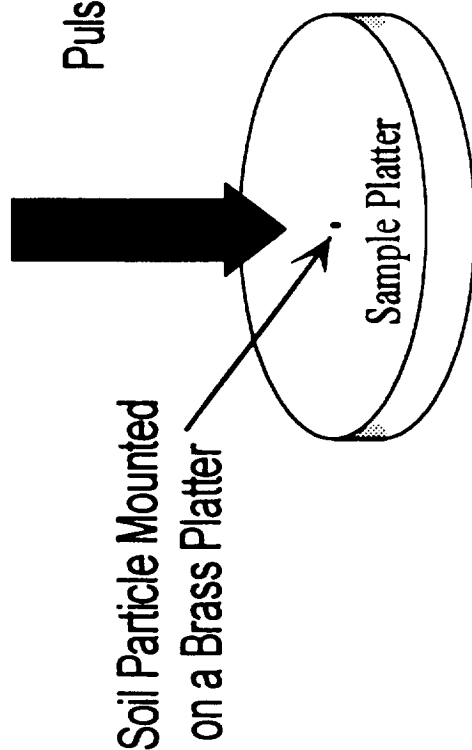


Figure 11: Principles of  $\mu\text{L}^2\text{MS}$  experiment

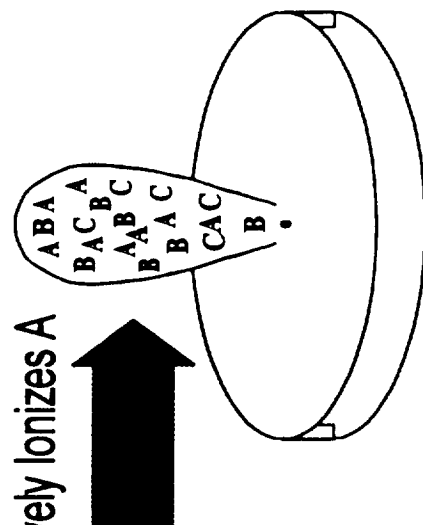
## (A) Laser Desorption of Neutral Molecules

## (B) Laser Ionization

Pulsed IR Laser Beam Focusable From  $50\ \mu\text{m}^2$  to  $5\ \mu\text{m}^2$

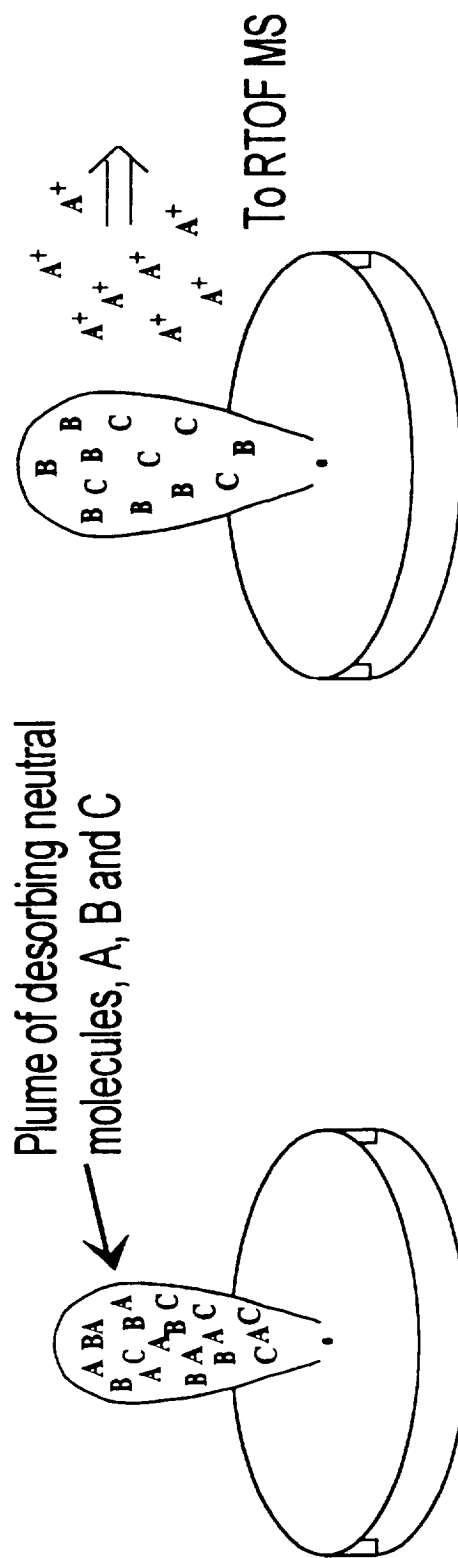


Pulsed UV Beam Selectively Ionizes A



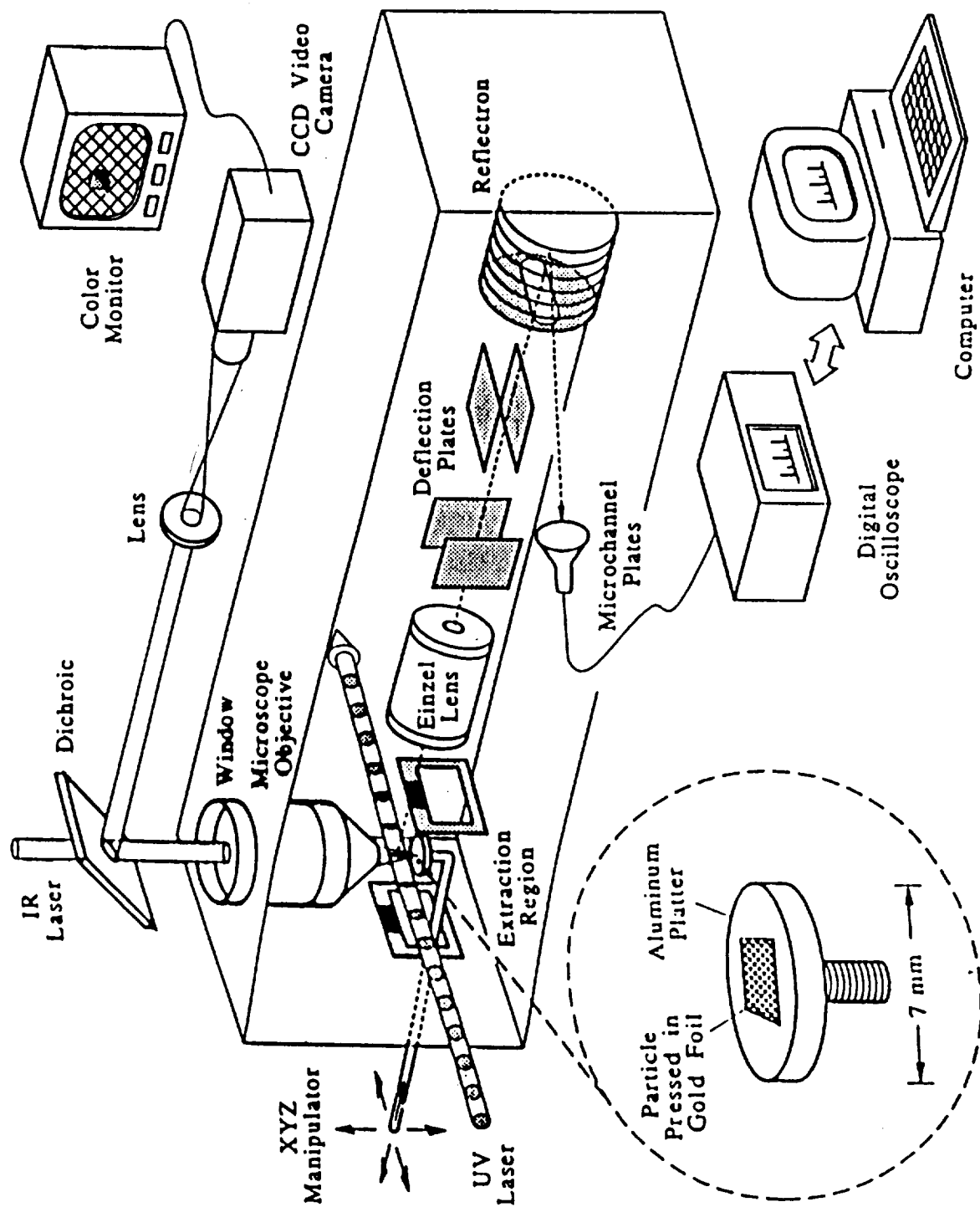
Plume of desorbing neutral

molecules, A, B and C



To RTOF MS

Figure 12: Schematic of  $\mu\text{L}^2\text{MS}$  instrument



**Figure 13: Mass spectra of Murchison using a CO<sub>2</sub> laser desorption source (10.6 μm)  
pre- and post- water extraction**

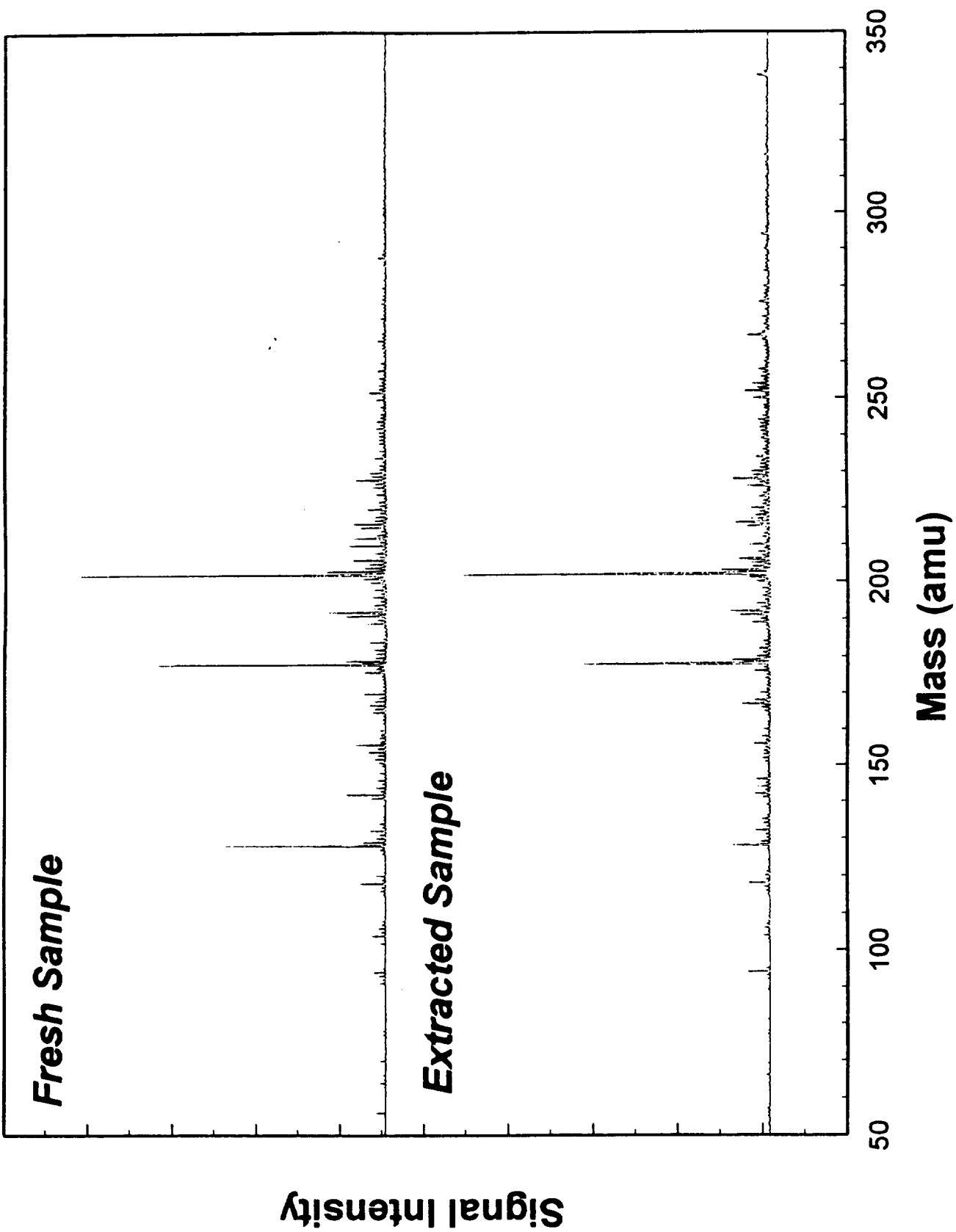
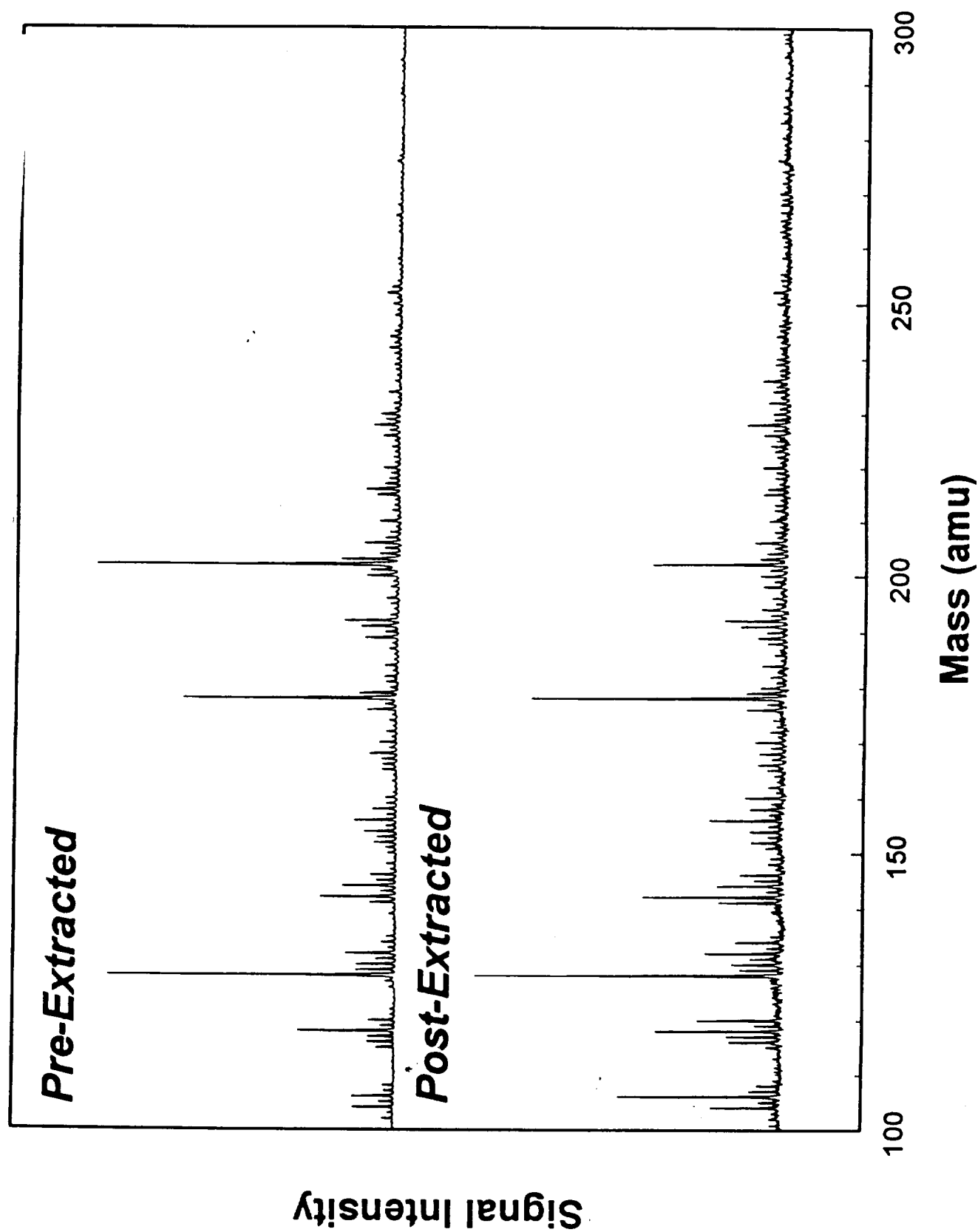


Figure 14: Mass spectra of Murchison using a CO<sub>2</sub> laser desorption source (10.6 μm)  
pre- and post- acid extraction



**Figure 15: Mass spectra of Murchison using an Er:YAG laser desorption source (2.94  $\mu\text{m}$ )  
pre- and post- acid extraction**

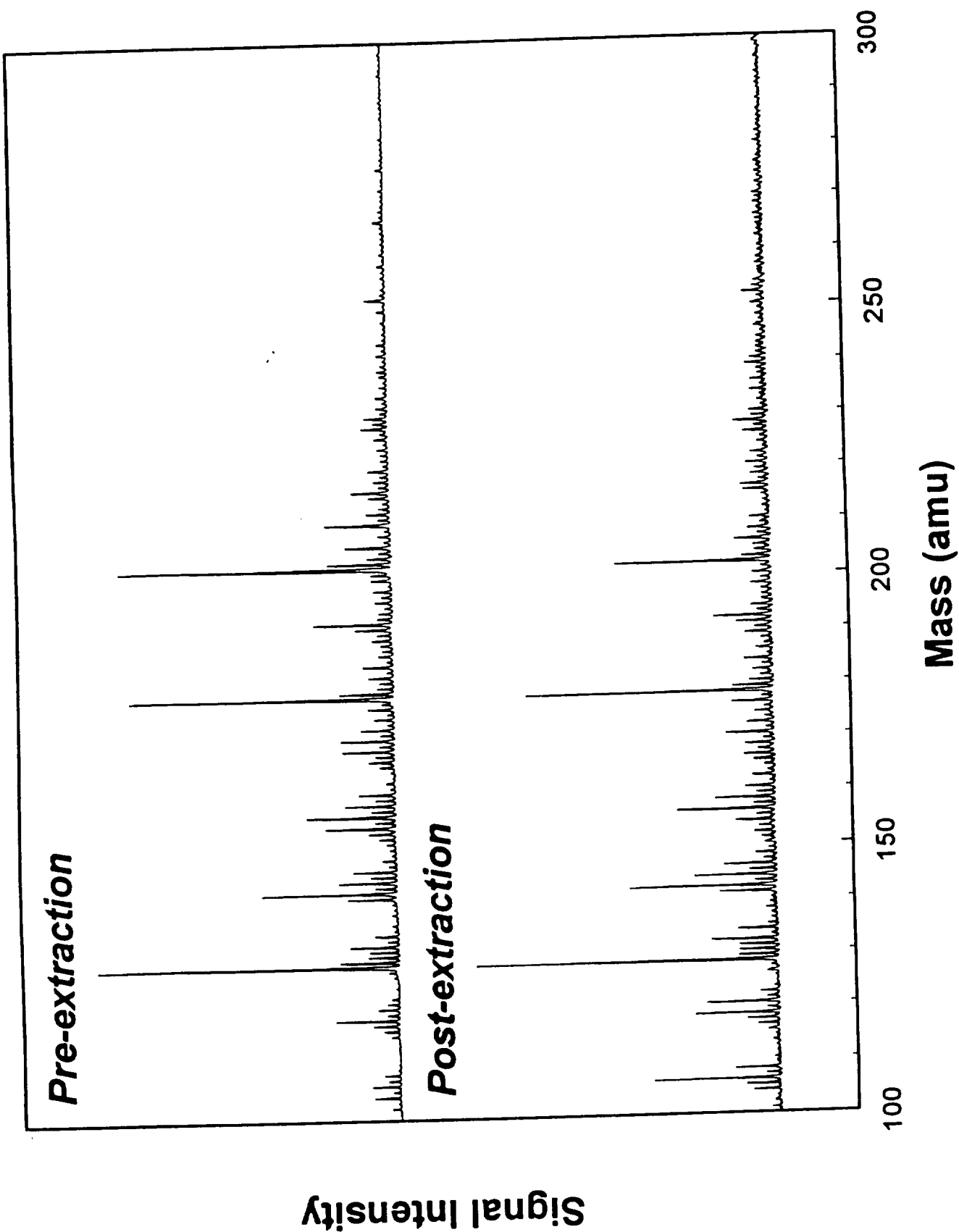
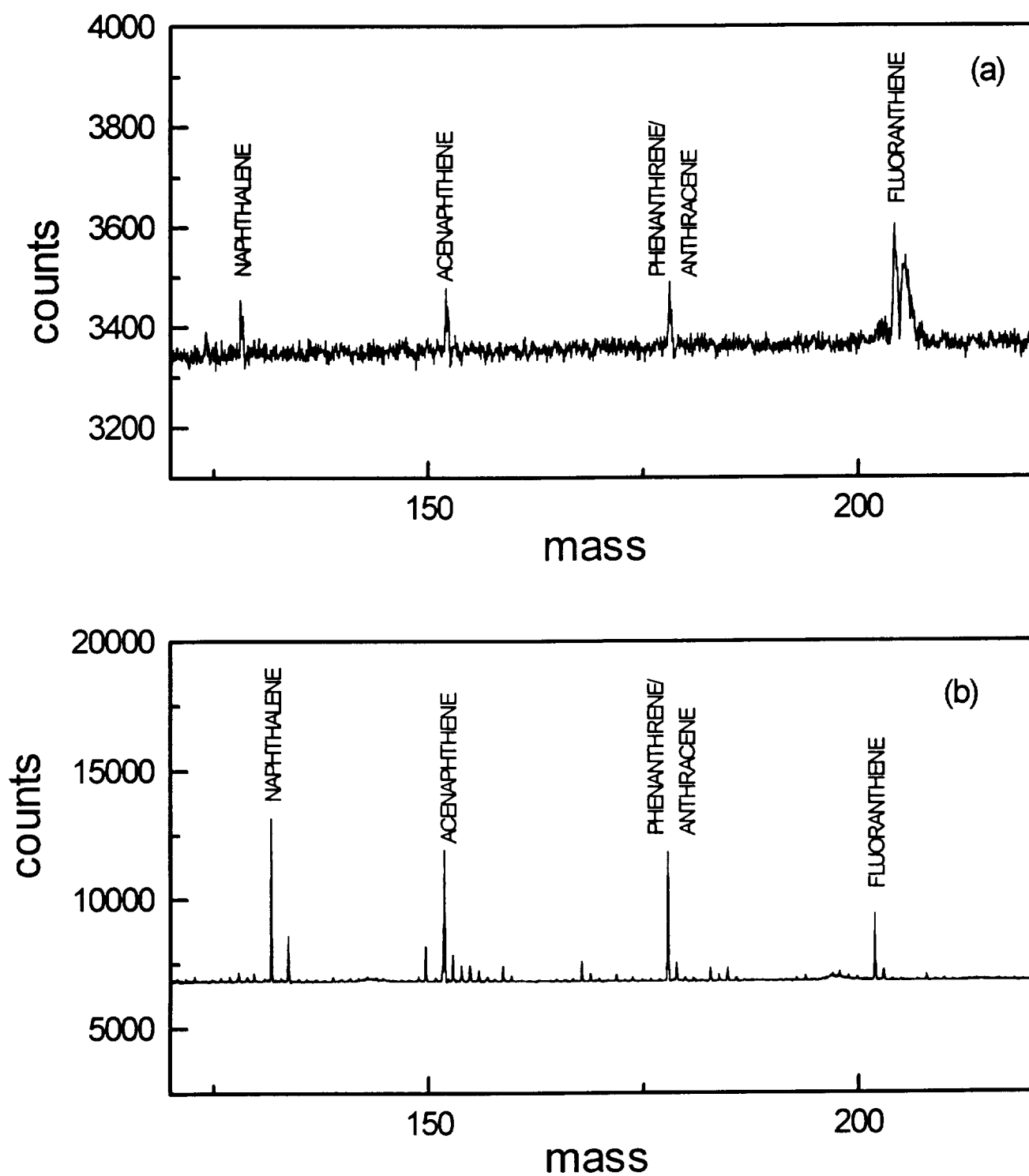
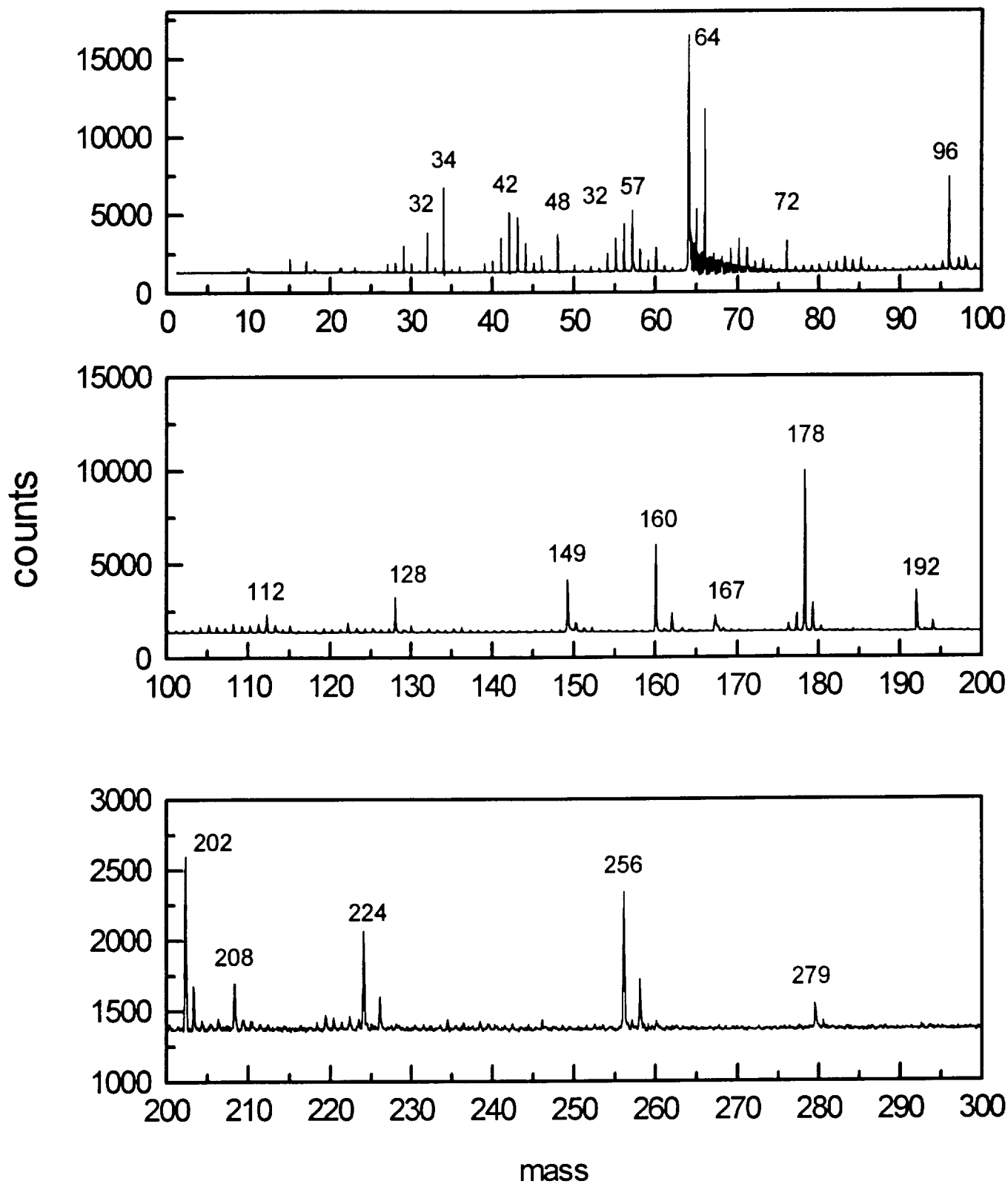


Figure 16



118-nm ionization / laser desorption mass spectra of a PAH mixture in toluene (1mg/ml). 12  $\mu$ l were dropped on  $\text{CaCO}_3$  powder and this was pressed on an In foil for analysis : a) 355 nm laser desorption, b) 532 nm desorption.

Figure 17a



118-nm ionization / 532-nm laser desorption mass spectrum of a fragment of the C2-Murchinson meteorite: a) full scale, b) expanded scale.

Figure 17b

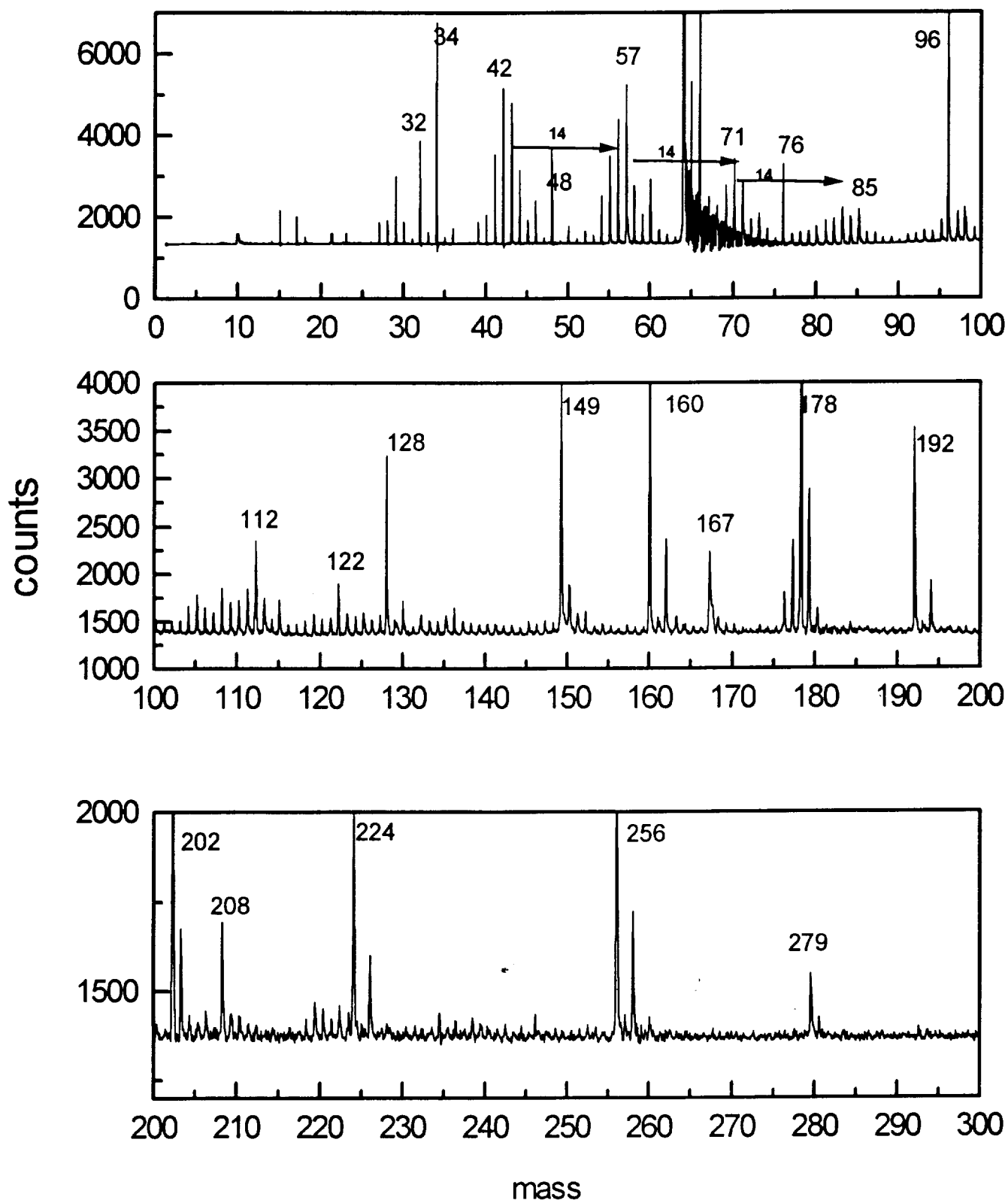
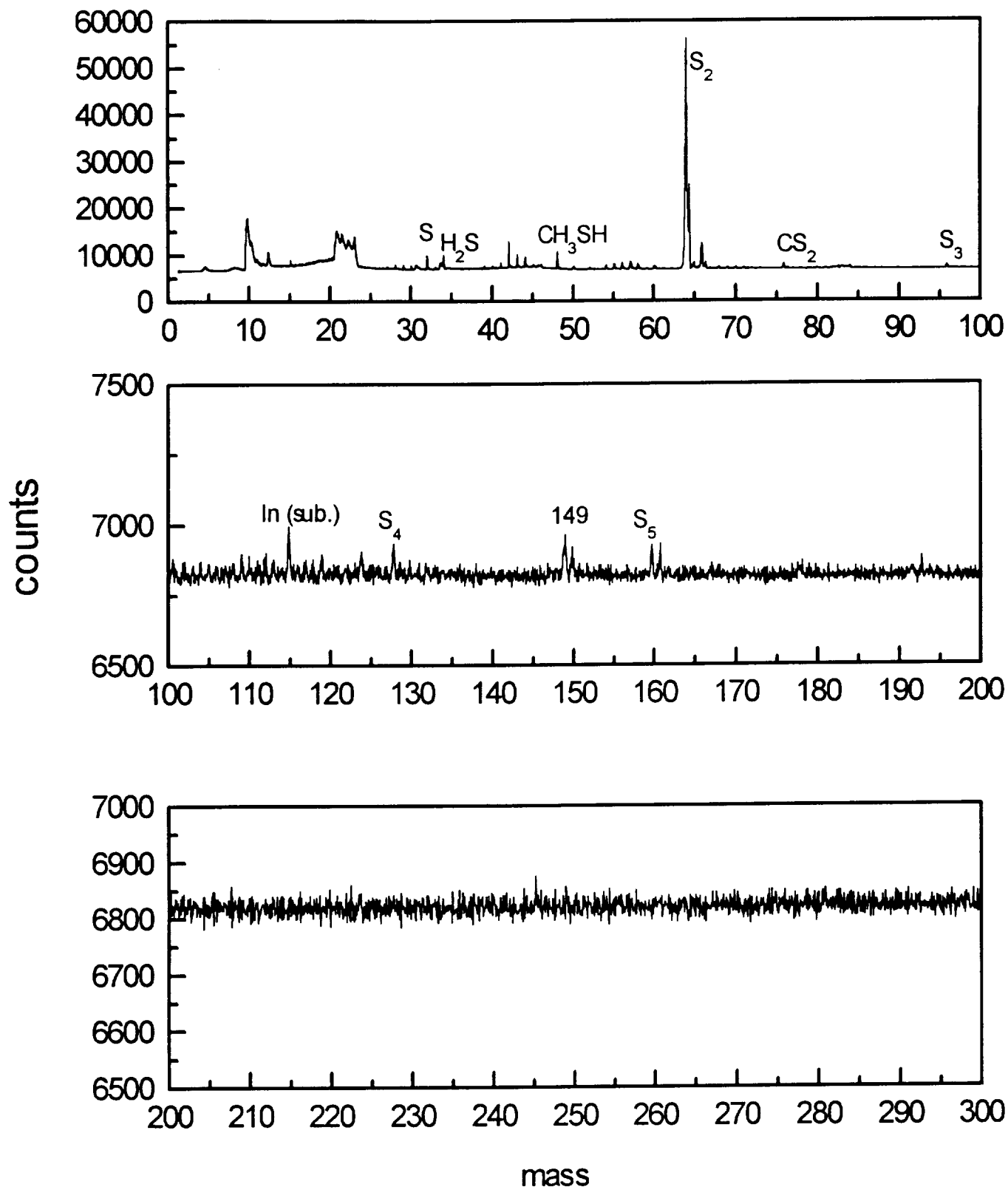


Figure 18a



118-nm ionization / 532-nm laser desorption mass spectrum of a fragment of the CV3 meteorite: a) full scale, b) expanded scale for the low mass range region. The wide peaks appearing at low mass are due to direct ions produced in the desorption step.

Figure 18b

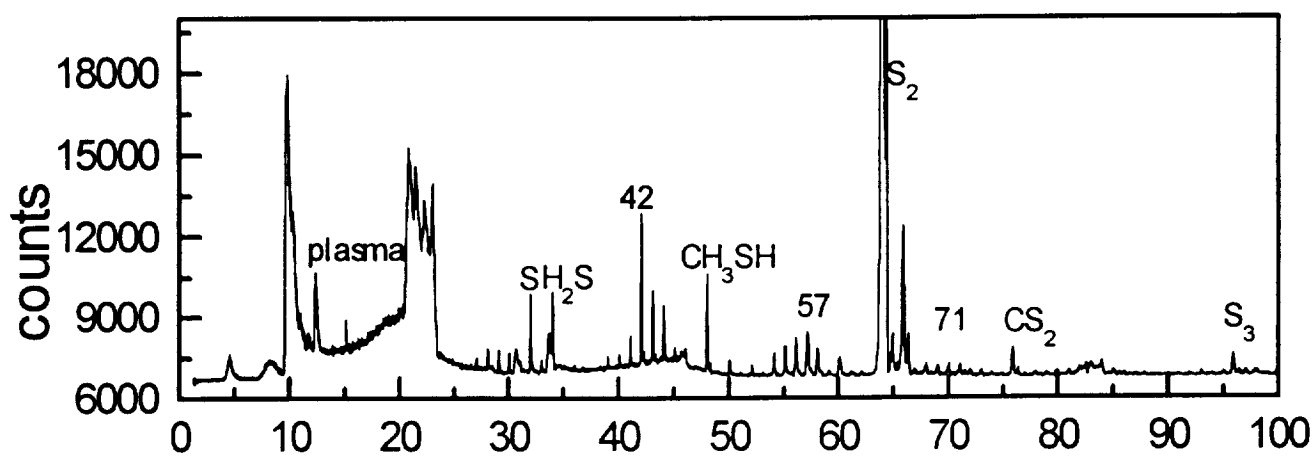
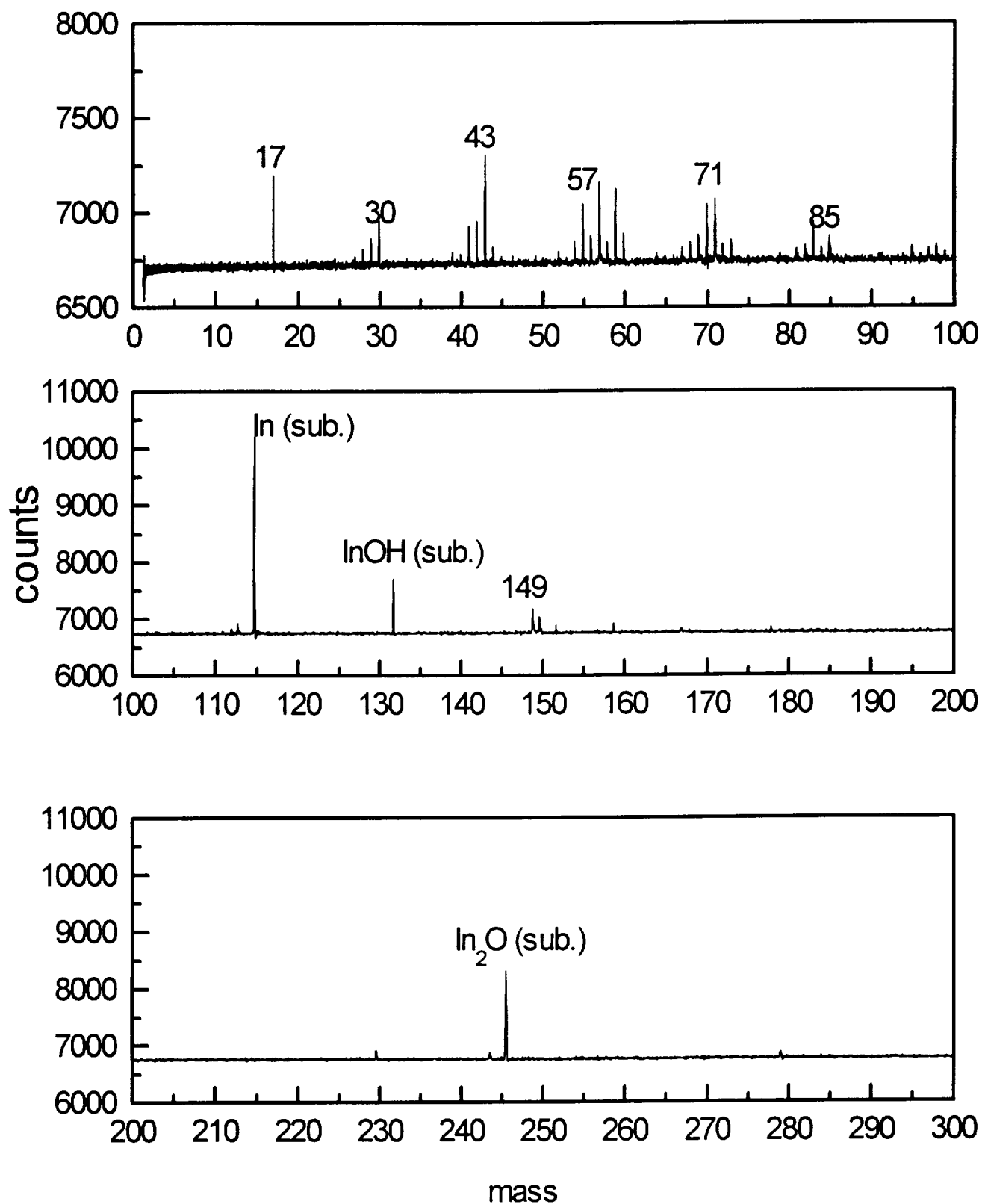
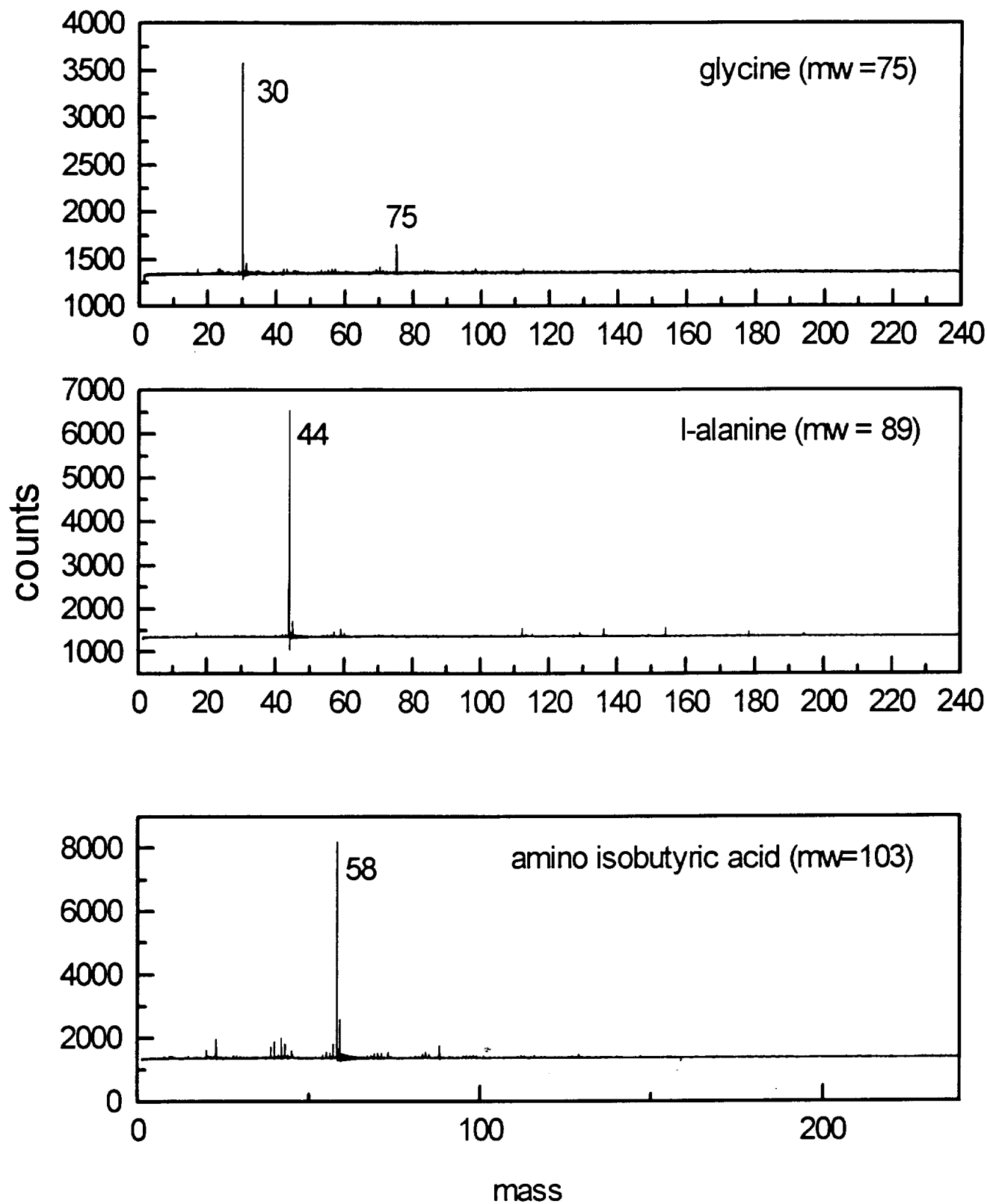


Figure 19



118-nm ionization / 532-nm laser desorption mass spectrum of a fragment of terrestrial coal. The fragment, as were the meteorite samples, was pressed into a In foil for the analysis.

Figure 20a



Sequence of 118-nm ionization mass spectra produced by a selected set of amino acids.

Figure 20b

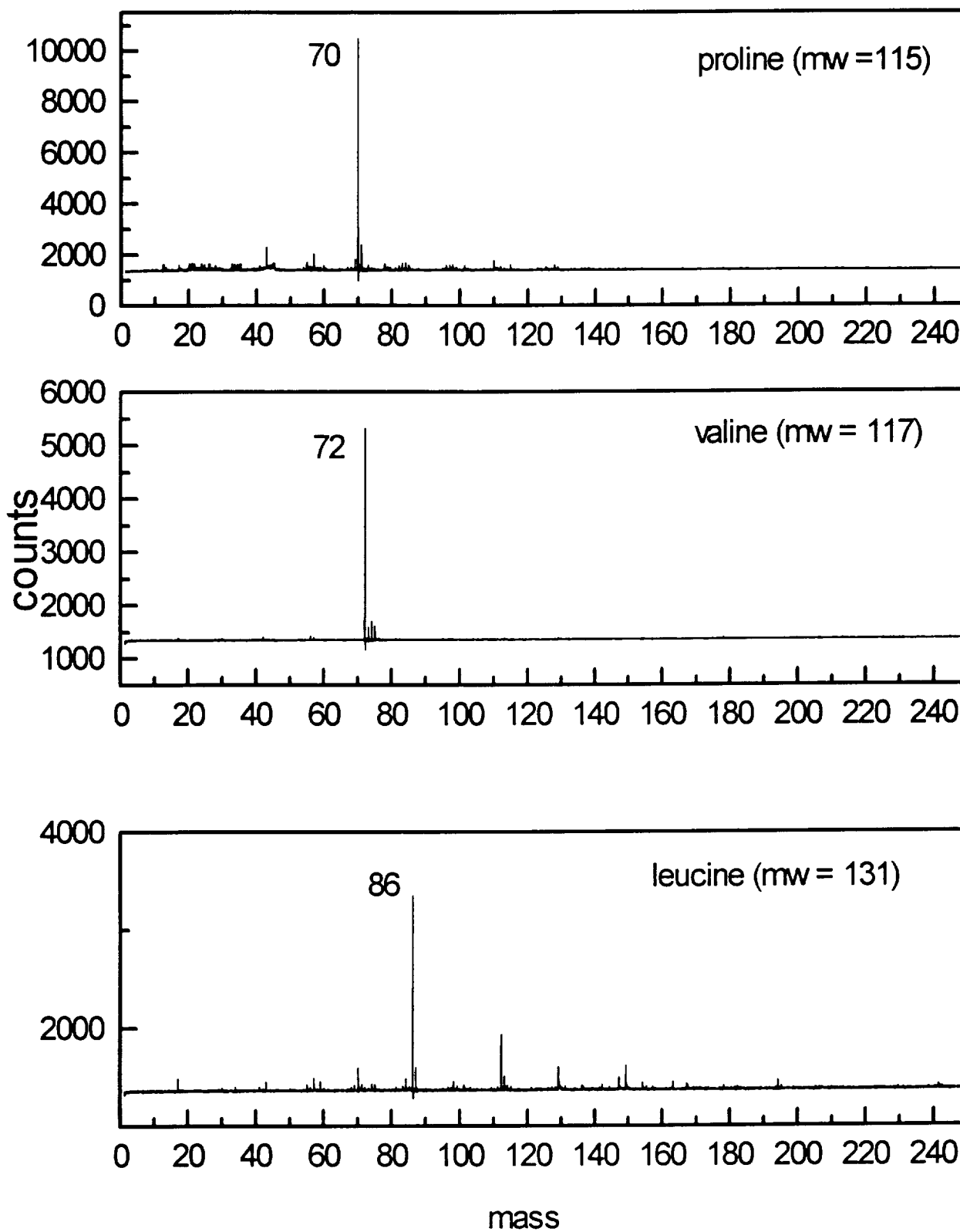


Figure 20c

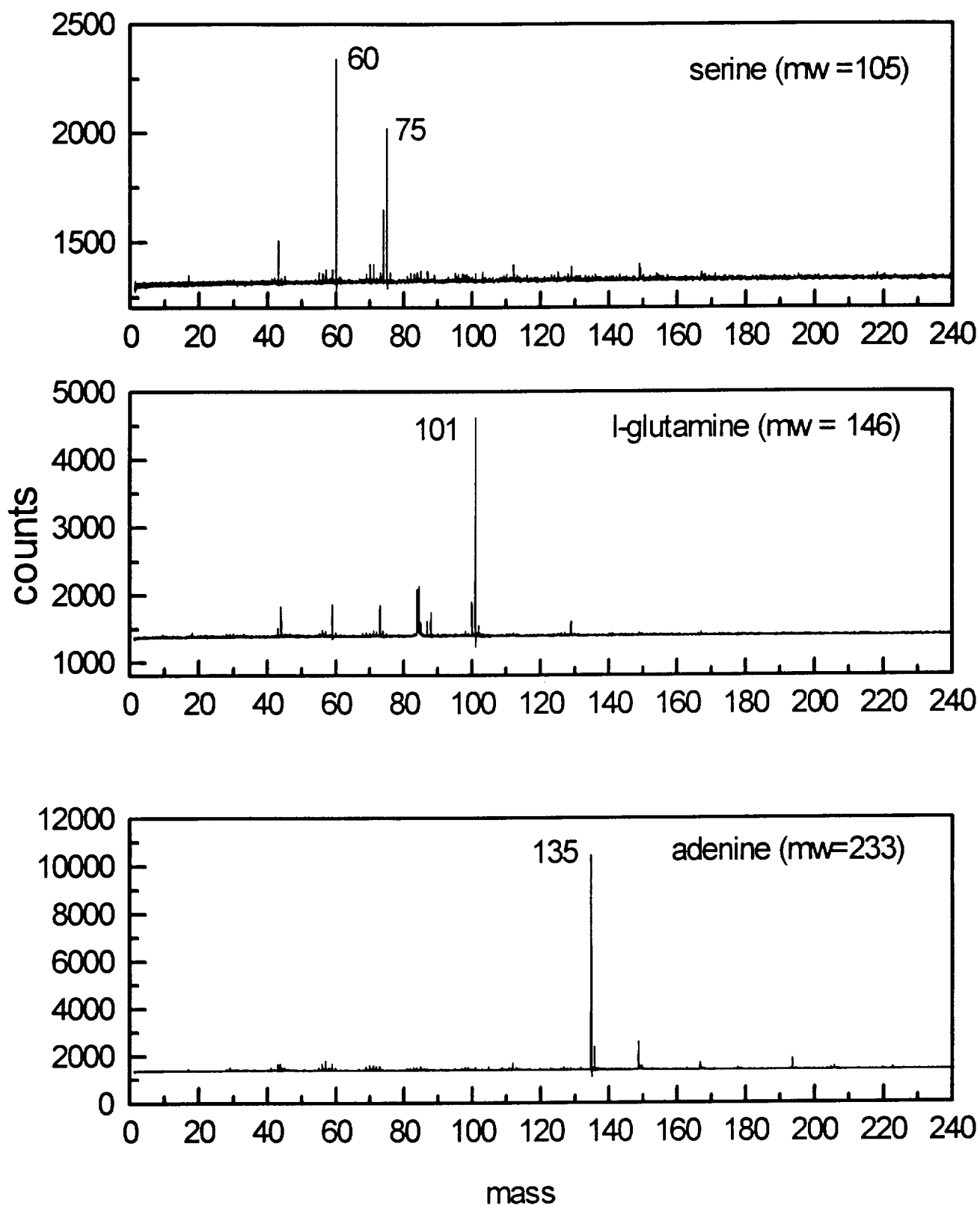
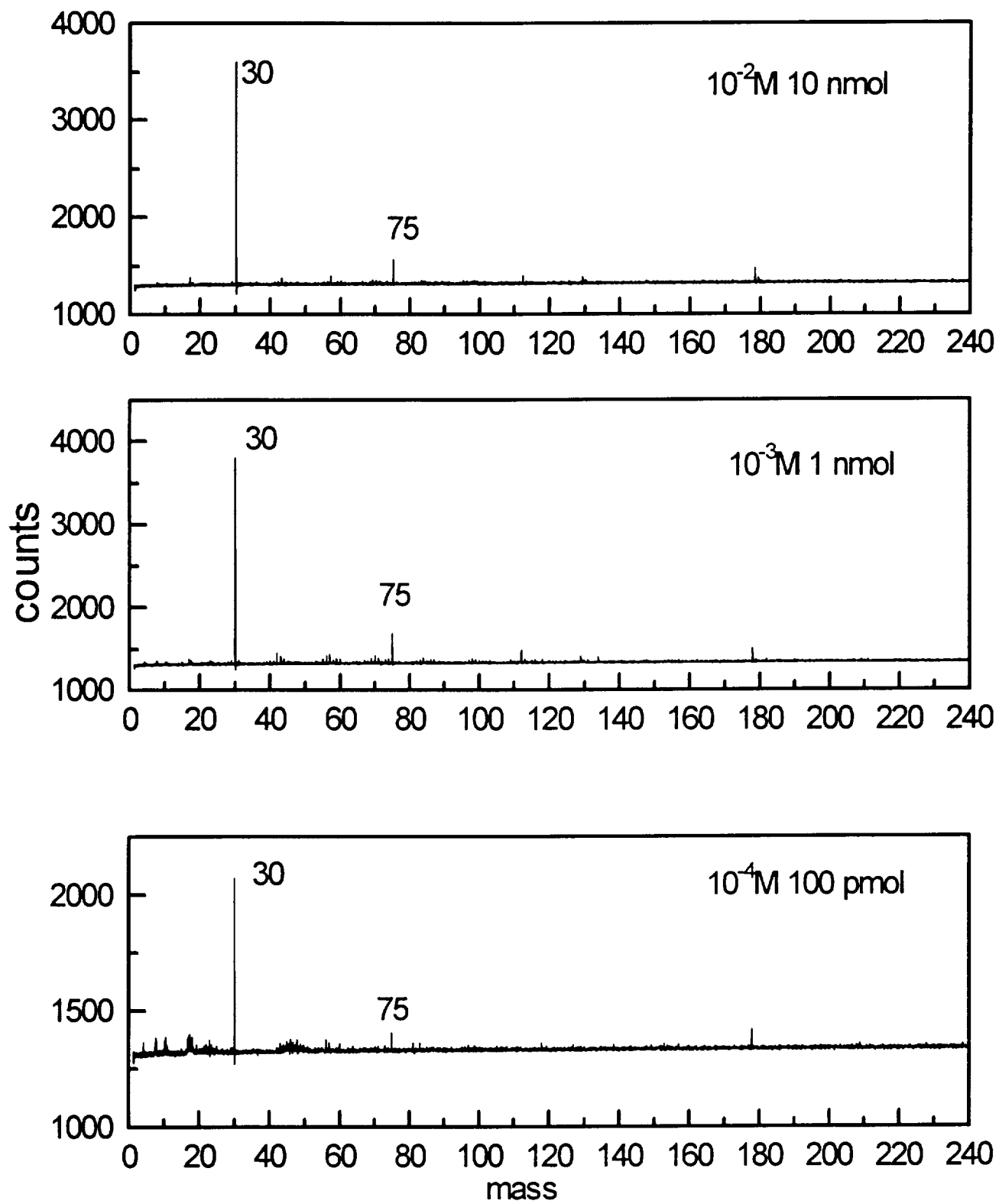
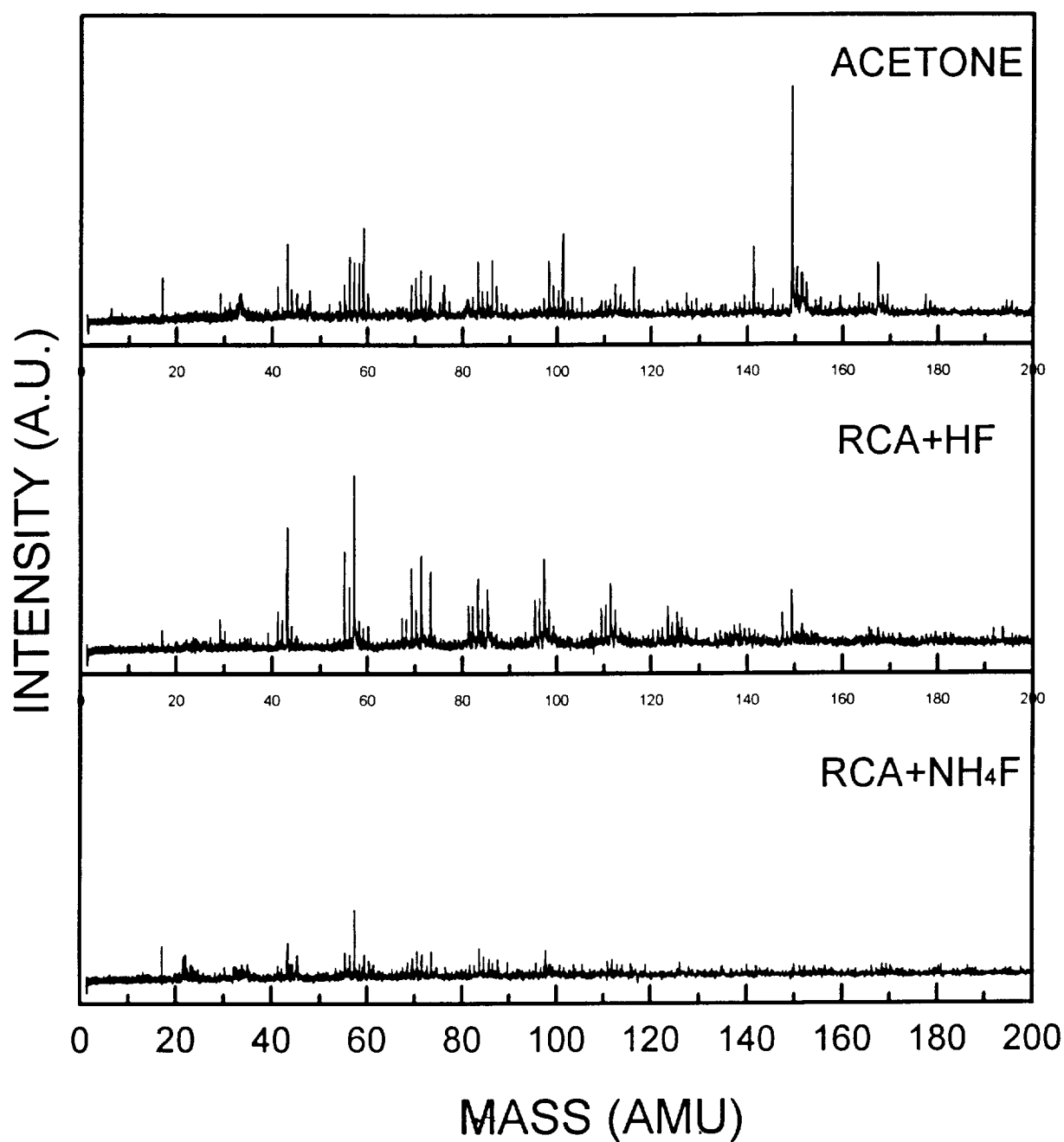


Figure 21



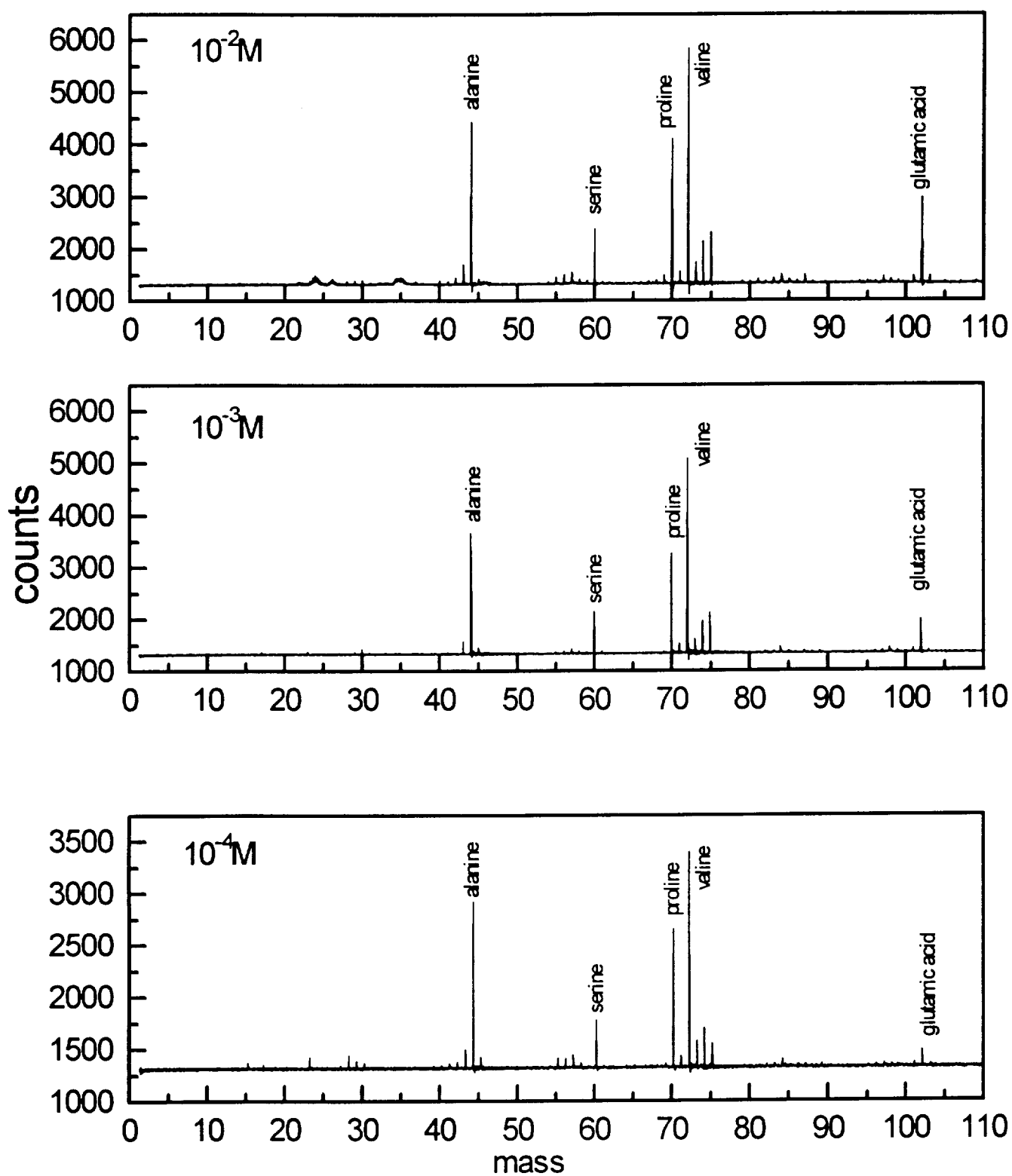
Sequence of spectra produced by glycine solutions of decreasing molarity.

Figure 22



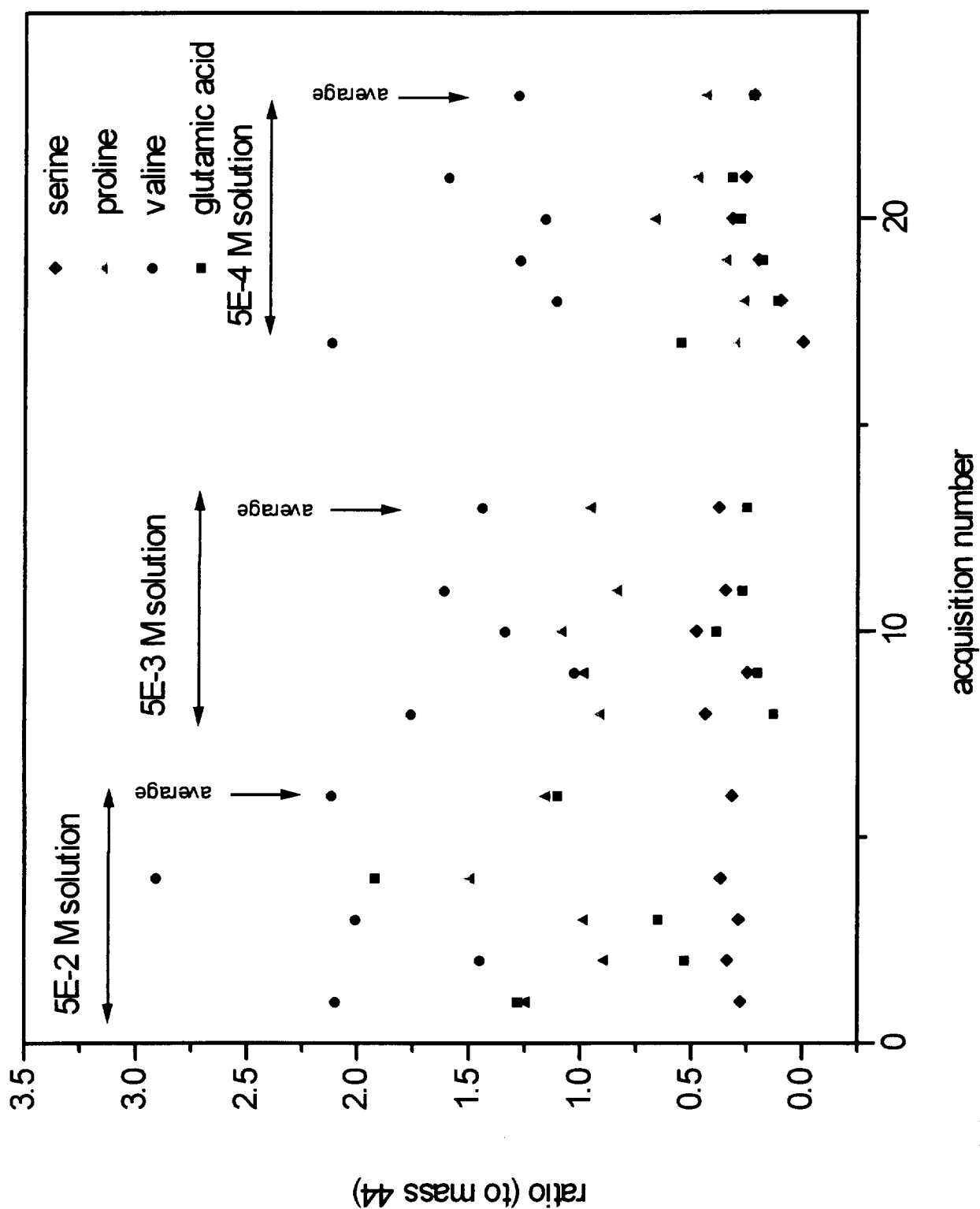
Effect of different cleaning procedures on a silicon substrate. RCA + HF and RCA + NH<sub>4</sub>F are standard cleaning processes used in microelectronics fabrication ( see for example "*Handbook of semiconductor cleaning technology: science, technology, and application*", W. Kern ed., Noyes Pub., 1993 Park Ridge, N. J., USA).

Figure 23



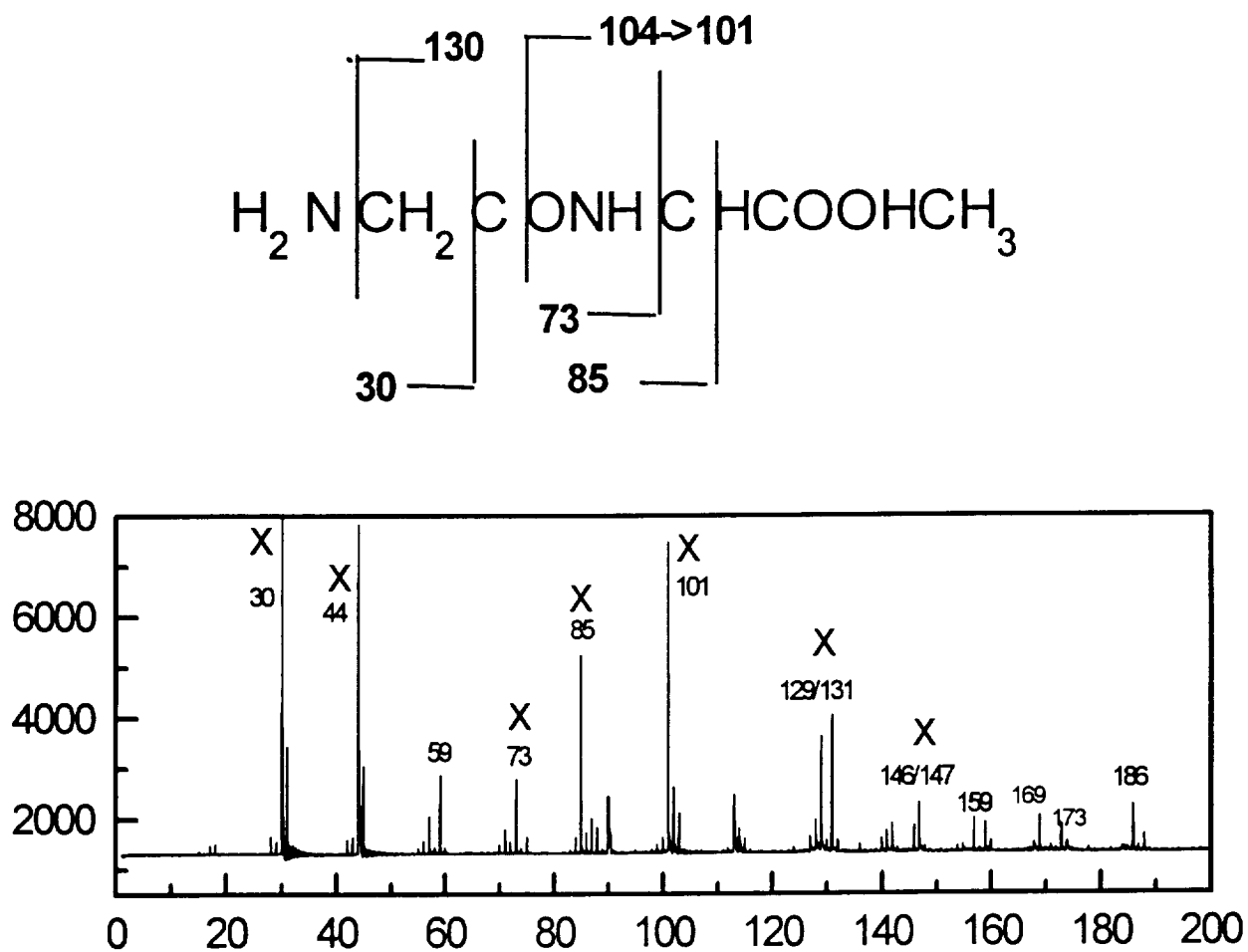
Sequence of spectra produced by solutions of 5 amino acids with decreasing molarity.

Figure 24



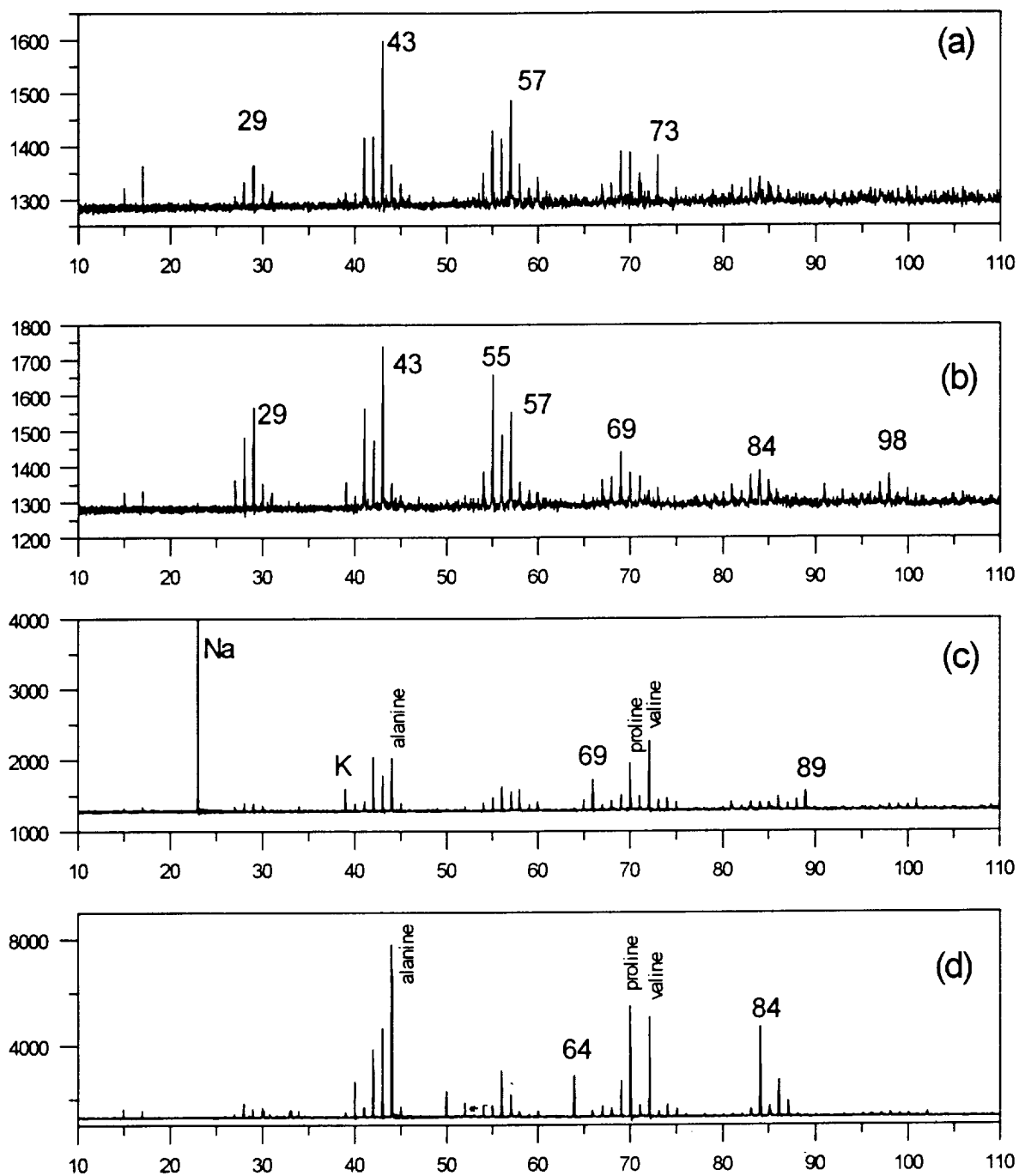
Ratio between the base-peak intensity of different amino acids and l-alanine (m=44). Each group represents acquisitions from different samples prepared using a solution with given molarity. The average values for each molarity are also reported.

Figure 25



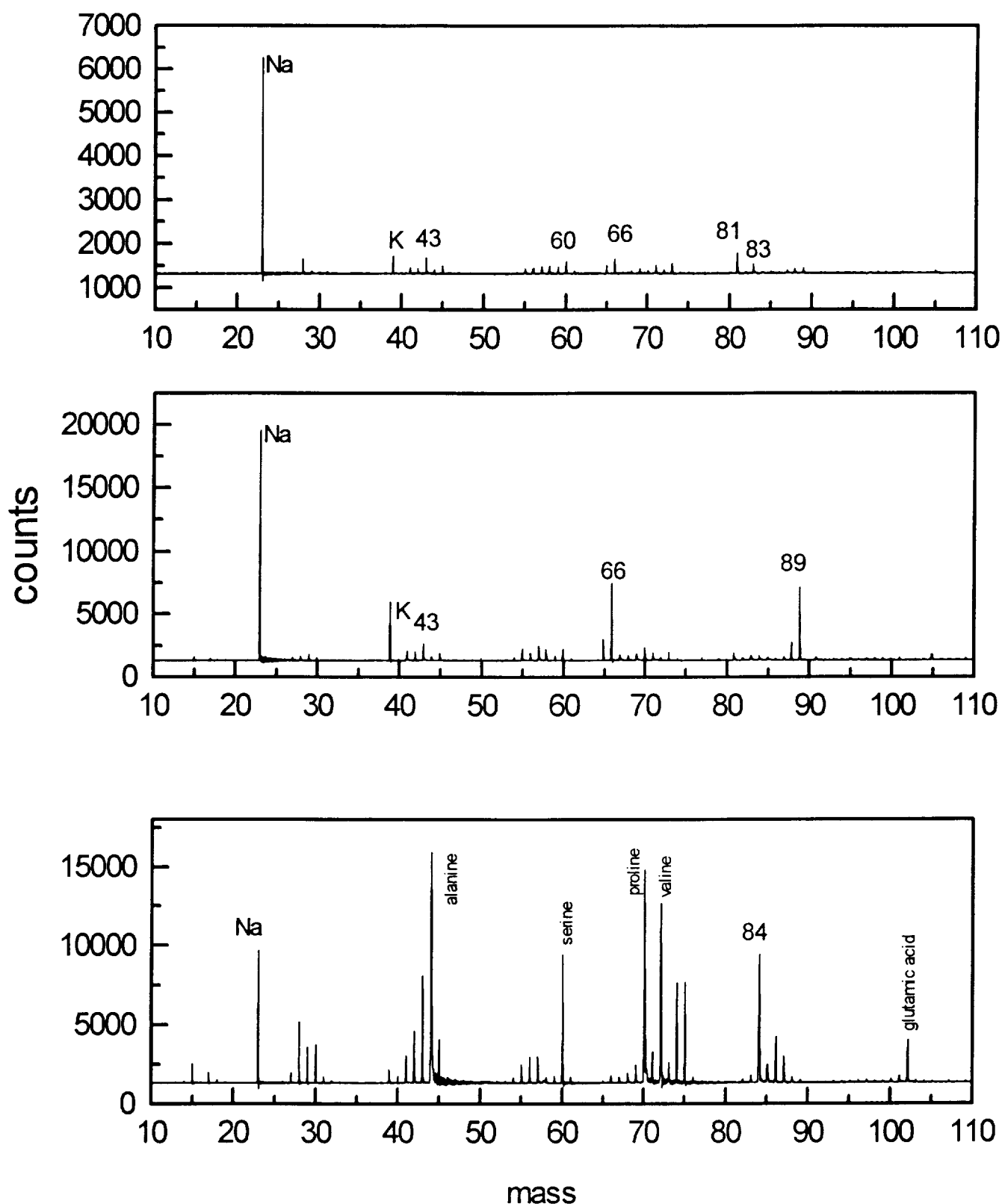
118-nm ionization mass spectrum of glycine-alanine. The molecular structure and possible fragmentation paths are given above. The marked peaks correspond to possible fragments. Loss or addition of an H is typical in 118-nm ionization mass spectra.

Figure 26



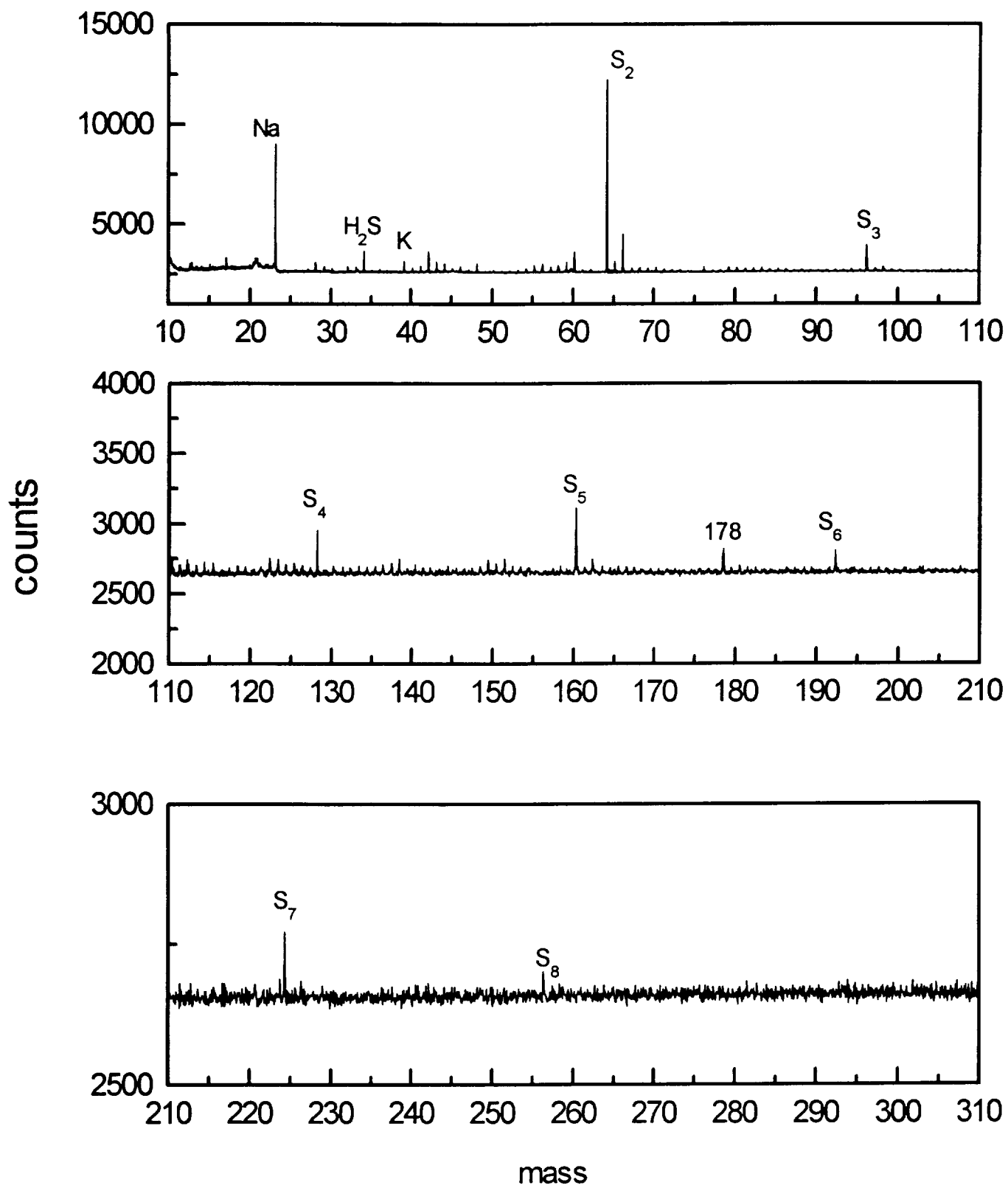
Results of the tests performed with a specific experimental set-up using a simulated sample (extraction conditions are given in the text): a) water circulated in the extraction column; b) extraction performed on pure SiO<sub>2</sub> powder; c) extraction of SiO<sub>2</sub> powder dosed with a known amount of amino acids; d) SiO<sub>2</sub> powder after extraction.

Figure 27



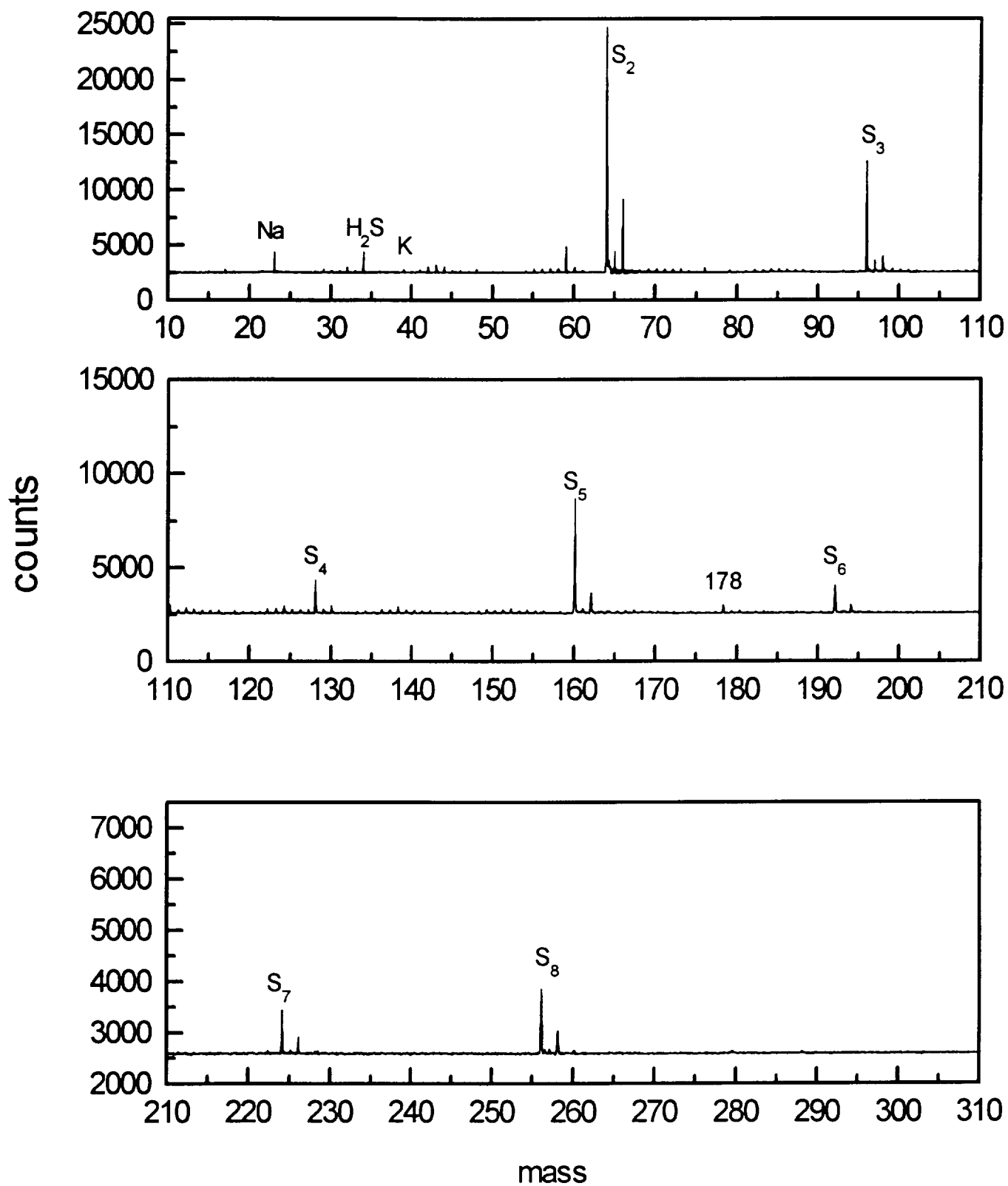
Results of the tests performed with a modified experimental set-up using a simulated sample (extraction conditions are given in the text): water circulated in the extraction column (top) ; extraction performed on pure  $\text{SiO}_2$  powder (middle); extraction of  $\text{SiO}_2$  powder dosed with a known amount of amino acids (bottom).

Figure 28



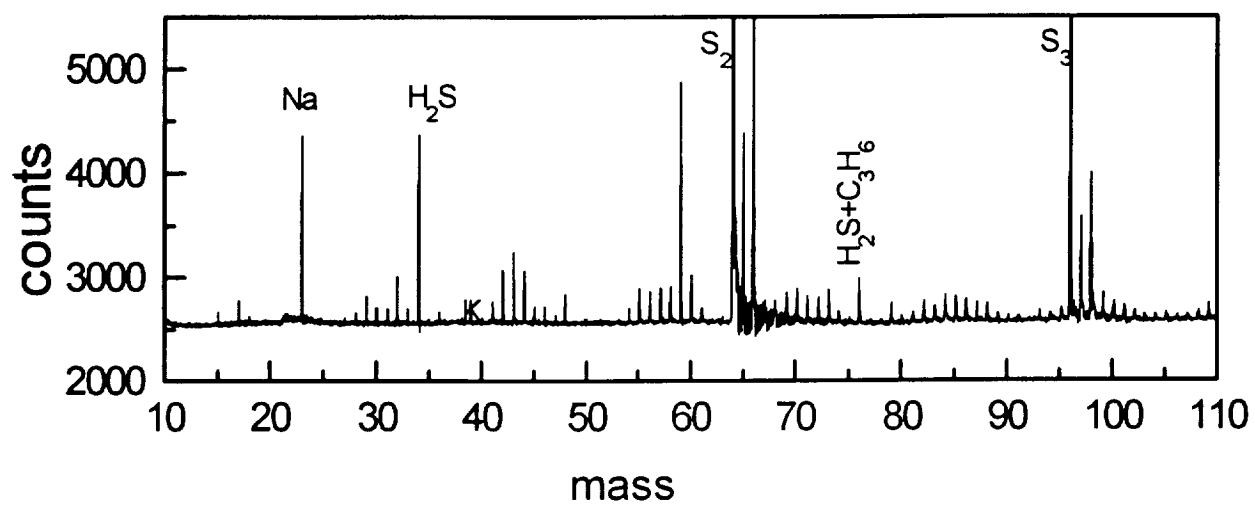
Extraction of Murchinson meteorite.

Figure 29a

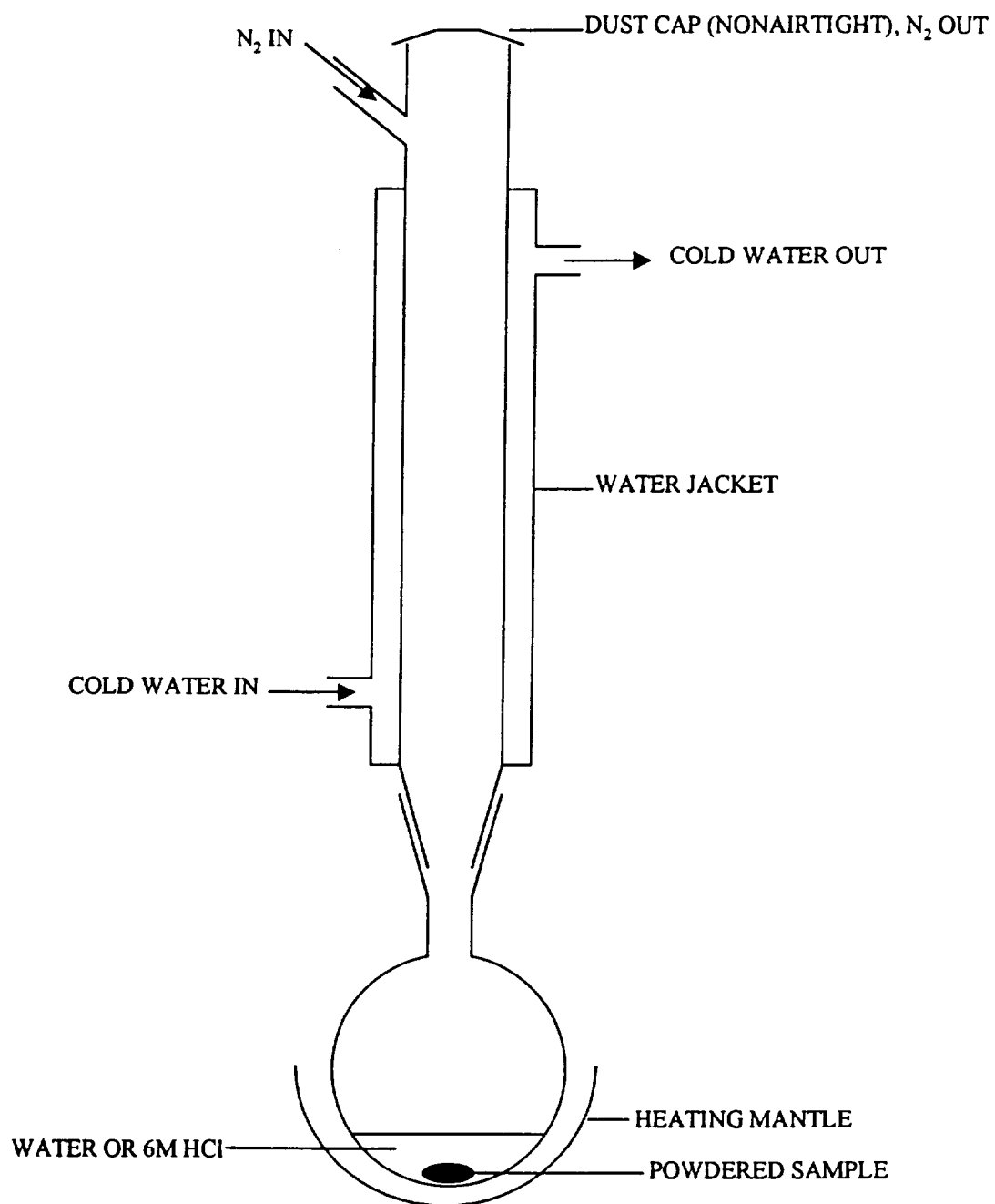


Extraction of Murchinson meteorite.

Figure 29b



**Figure 30: Amino acid extraction apparatus**



**Figure 31: Derivatization reaction of amino acids with fluorescein isothiocyanate (FITC)**

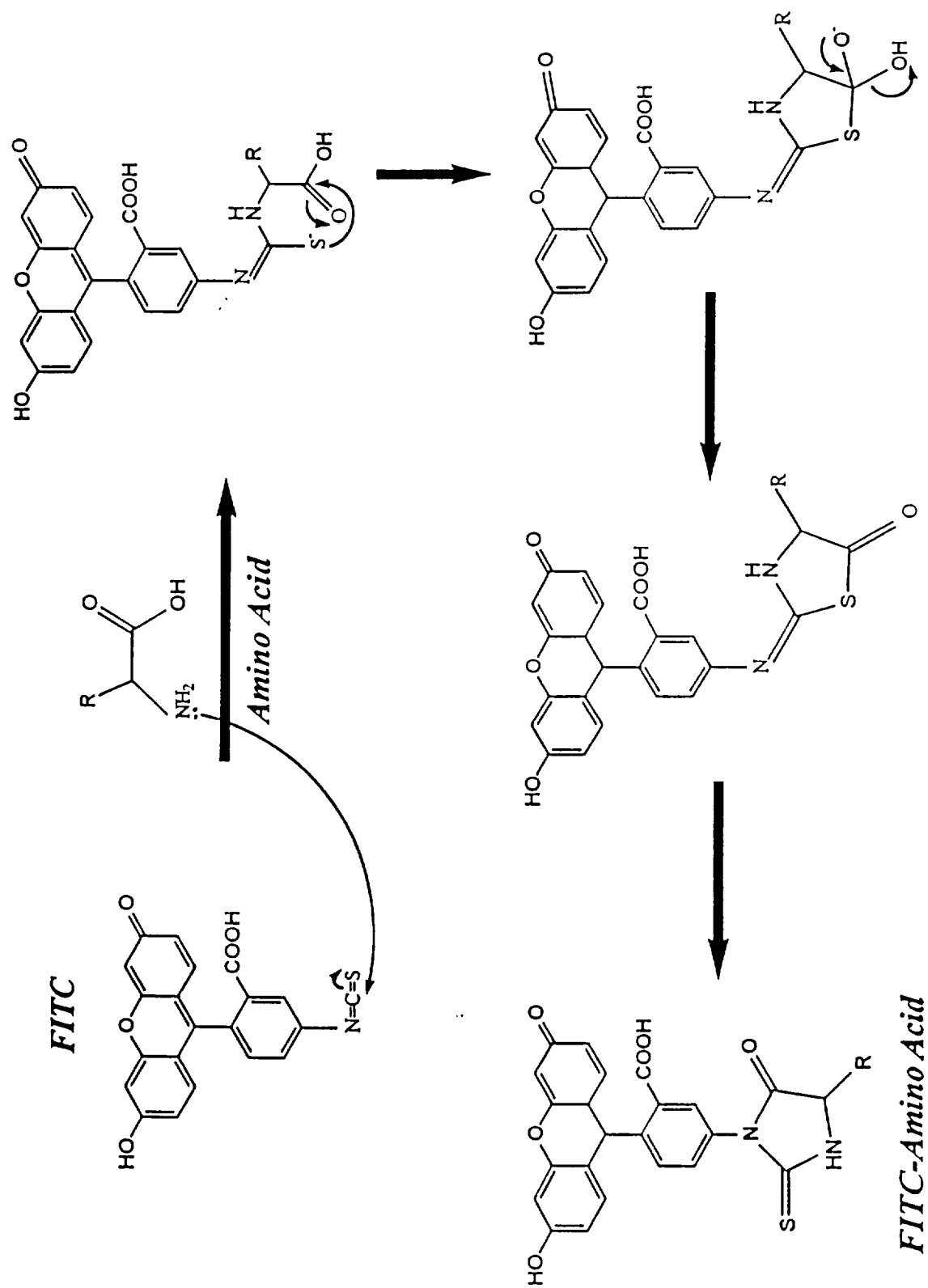
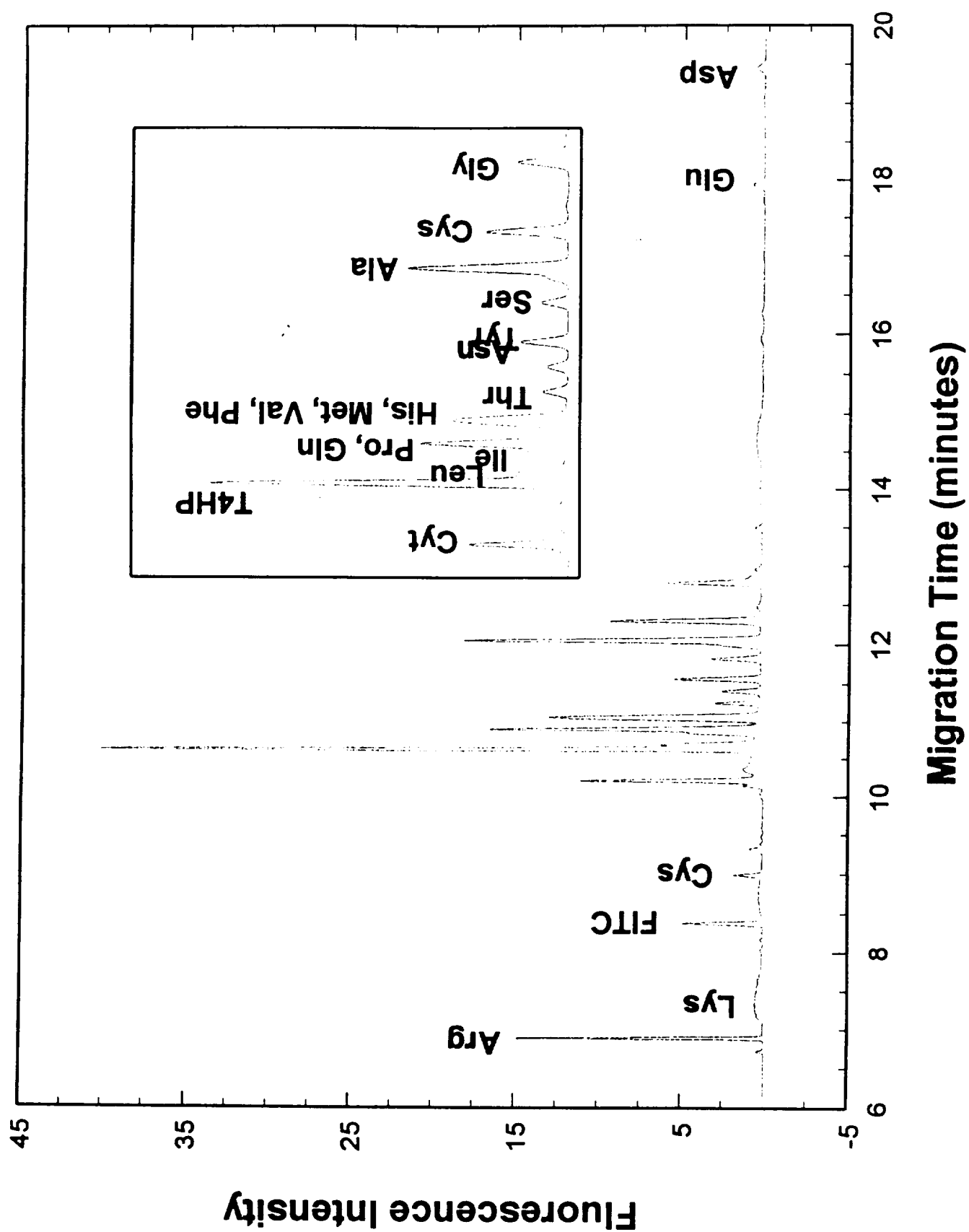


Figure 32: Separation of a laboratory solution of 21 amino acids



# Table 1: Solubilities of some of the PAHs

Molecular Species (AccuStandard) <sup>a</sup>	Formula	Mass (amu)	Solubility (mg/kg @ 25 C) <sup>b</sup>	Concentration (Becker et al., 1997) <sup>c</sup>	Precipitation (mg/kg) <sup>d</sup>
Naphthalene	C <sub>10</sub> H <sub>8</sub>	128	31.69 ± 0.23	0.063	-none-
Acenaphthene	C <sub>12</sub> H <sub>10</sub>	154	0.453	0.063	-none-
Fluorene	C <sub>13</sub> H <sub>10</sub>	166	1.685 ± 0.005	0.063	-none-
Acenaphthylene	C <sub>13</sub> H <sub>12</sub>	168	16.1	0.063	-none-
<i>Anthracene</i>	C <sub>14</sub> H <sub>10</sub>	178	0.0446 ± 0.0002	0.063	0.0184 (29.2%)
<i>Phenanthrene</i>	C <sub>14</sub> H <sub>10</sub>	178	1.002 ± 0.011	0.063	-none-
<i>Pyrene</i>	C <sub>16</sub> H <sub>10</sub>	202	0.132 ± 0.001	0.063	-none-
<i>Fluoranthene</i>	C <sub>16</sub> H <sub>10</sub>	202	0.206 ± 0.002	0.063	-none-
<i>Chrysene</i>	C <sub>18</sub> H <sub>12</sub>	228	0.0018 ± 0.0001	0.063	0.0612 (97.1%)
<i>Benzo(a)anthracene</i>	C <sub>18</sub> H <sub>12</sub>	228	0.0094	0.063	0.0536 (85.1%)
<i>Benzo(b)fluoranthene</i>	C <sub>20</sub> H <sub>12</sub>	252	0.0020	0.063	0.0610 (96.8%)
<i>Benzo(k)fluoranthene</i>	C <sub>20</sub> H <sub>12</sub>	252	0.0038	0.063	0.0592 (89.2%)
<i>Benzo(a)pyrene</i>	C <sub>20</sub> H <sub>12</sub>	252	0.0038 ± 0.00031	0.063	0.0627 (99.5%)
<i>Benzo(g,h,i)perylene</i>	C <sub>22</sub> H <sub>12</sub>	276	0.0003	0.063	0.0627 (99.5%)
<i>Indeno(1,2,3-cd)pyrene</i>	C <sub>22</sub> H <sub>12</sub>	276	≤ 0.01	0.063	0.053 (>84.1%)
<i>Dibenzo(a,h)anthracene</i>	C <sub>22</sub> H <sub>14</sub>	276	≤ 0.01	0.063	0.053 (>84.1%)

- (a) PAH Standard "Accustandard" (Accustandard, 25 Science Park, New Haven, CT 06511). This was the PAH standard used by Becker et al. [Becker, L.; Glavin, D. P.; Bada, J. L.; *Geochim. Cosmochim. Acta*, **67**, 475 (1977)]. PAH Species in *italic* were observed in ALH84001 [McKay, D. S.; Gibson Jr., E. K.; Thomas-Kepra, K. L.; Vali, H.; Romanek, C. S.; Clemett, S. J.; Chillier, X. D. F., Maochling, C. R.; Zare, R. N.; *Science*, **273**, 924 (1986)].
- (b) Experimentally measured solubility of PAHs:  
MacKay, D. S.; Shiu, W. Y.; *J. Chem. Eng. Data*, **22**, 399 (1977)  
Schwarz, F. P.; *J. Chem. Eng. Data*, **22**, 273 (1977)  
May, W. E.; Brown, J. M.; Chesler, S. N. Guenther, F.; Hilpert, L. R.; Hertz, M. S.; Wise, S. A.; in "Polynuclear Aromatic Hydrocarbons" (Jones, P. W. and Leber, P., eds.), P. 411, Ann Arbor Science Publ., Ann Arbor, Michigan, 1979  
May, W. E.; Wasik, S. P.; Freeman, D. M.; *Anal. Chem.*, **50**, 997 (1978)  
Walters, W. R.; Luthy, R. G.; *Environ. Sci. Technol.*, **18**, 395 (1984)
- (c) Concentration of each PAH stated to have be dissolved in a solution of doubly distilled water in the "Carbonate Extraction Experiment" of Becker et al. [Becker, L.; Glavin, D. P.; Bada, J. L.; *Geochim. Cosmochim. Acta*, **67**, 475 (1997)].
- (d) Quantity of each PAH that could not have been dissolved and thus precipitated out, and its percentage relative to the total quantity of that PAH added. In all cases the experimentally measured solubility of each PAH assumes that it is the only aromatic species present, furthermore it is assumed that the PAH is given sufficient time to solubilize

**Table II: Tentative identification of the major peaks present in spectrum of Fig. 2**

MASS	COMPOUND/FRAGMENT
32	S
34	SH <sub>2</sub>
42	C <sub>3</sub> H <sub>6</sub>
48	CH <sub>3</sub> SH
57	C <sub>n</sub> H <sub>2n</sub> N +H or C <sub>n</sub> H <sub>2n</sub> NCO n=1
64	S <sub>2</sub>
71	C <sub>n</sub> H <sub>2n</sub> N +H or C <sub>n</sub> H <sub>2n</sub> NCO n=2
72	C <sub>4</sub> H <sub>10</sub> N, C <sub>3</sub> H <sub>6</sub> NO
76	CS <sub>2</sub>
85	C <sub>n</sub> H <sub>2n</sub> N +H or C <sub>n</sub> H <sub>2n</sub> NCO n=3
96	S <sub>3</sub>
112	C <sub>8</sub> H <sub>16</sub>
115	In (substrate)
122	C <sub>7</sub> H <sub>8</sub> NO
128	S <sub>4</sub>
149	Phthalates base peak, poly(dimethylsiloxane)
160	S <sub>5</sub>
167	Phthalate peak
178	Phenanthrene/anthracene, poly(dimethylsiloxane)
192	S <sub>6</sub> , C <sub>14</sub> H <sub>9</sub> CH <sub>3</sub>
202	Phthalate peak, pyrene/fluoranthene
208	poly(dimethylsiloxane)
224	S <sub>7</sub>
256	S <sub>8</sub>
279	Phthalate peak

**Table III:** Amino acids selected to assess 118-nm sensitivity. Their molecular weight and the base peak present in the 118-nm ionization spectrum are also reported.

AMINO ACID	MW	Base peak
Glycine	75	30
l-alanine	89	44
Amino isobutyric acid	103	58
l-serine	107	60 (75)
Proline	115	70
Valine	117	72
Leucine	131	86
L-glutamine	146	101
Adenine*phosphate salt	233	135*